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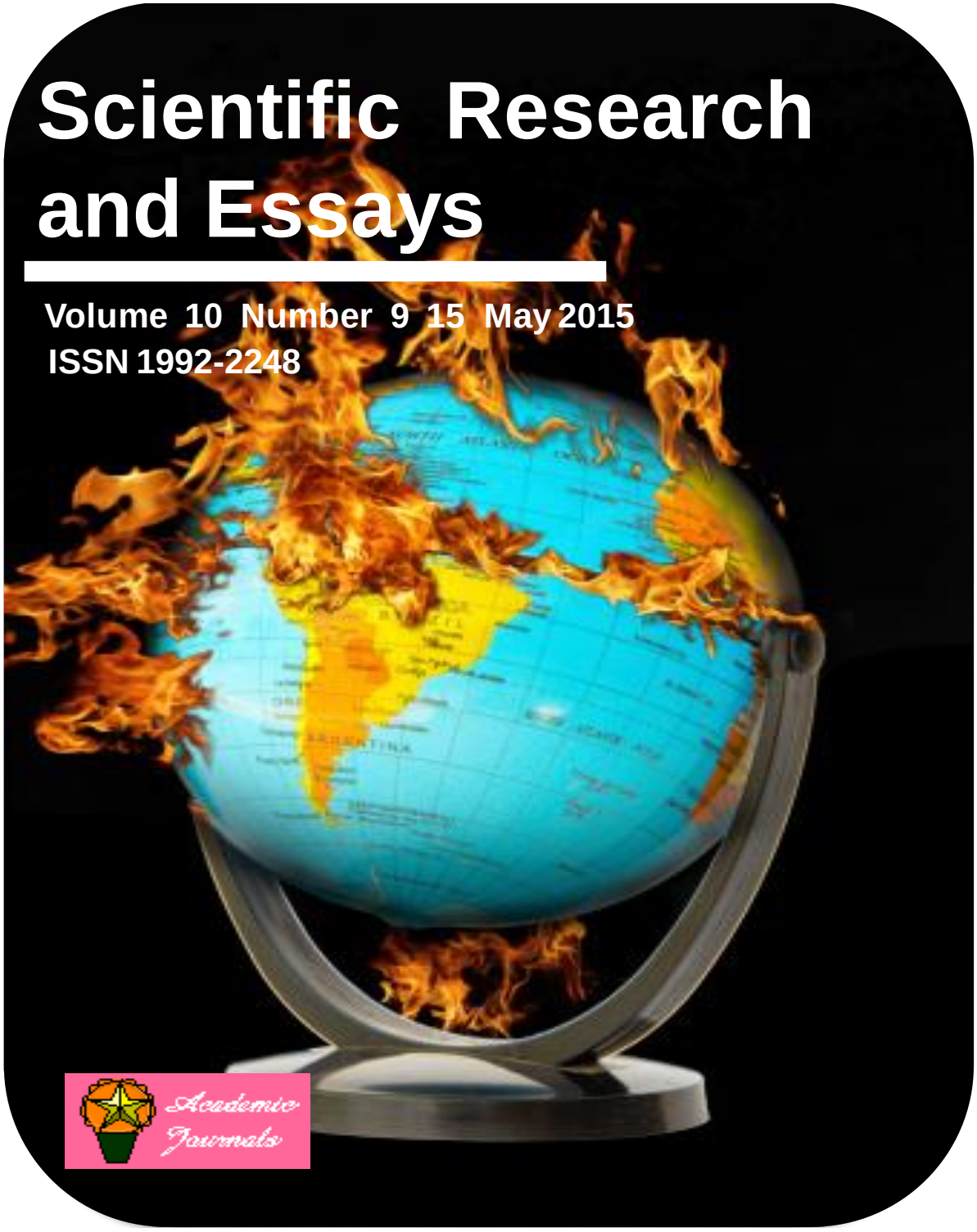

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Scientific Research and Essays

Full Length Research Paper

Evaluation for nutritive values and antioxidant activities of dried seablite (*Suaeda maritima*)

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The aims of these studies were to evaluate nutritive values of dried seablite (*Suaeda maritima*) and investigate its biological characteristics on health promotion, such as, antioxidant activities. Edible parts were collected, dried with solar stove, determined nutritive values, including proximal analysis of water content, crude protein, crude fat, dietary fiber, total ash content, carbohydrate, total calories, β-carotene, vitamin E, vitamin C, calcium, iron and sodium. The main of nutritive values and antioxidant activities of dried seablite were still remained, however, all of vitamin contents in dried seablite were vanished. In storage condition (sealed in plastic bag), nutritive value and antioxidant activities of dried seablite were remained after kept in 25°C for three months and not different when compared to former. In conclusion, dried seablite was still contained preferable nutritive values with antioxidant activities and non-toxic effect on Vero cell.

Key words: *Suaeda maritima*, seablite, food processing, shelf-life.

INTRODUCTION

Seablite (*Suaeda maritima*) is a salt marsh plant growing in mangrove forest. Its young leaves can be used as fresh vegetable or cooked. The cooked seablite is quite salty, so it should be cooked with other types of vegetable to reduce salty taste (Tanaka, 1976). Local people in Samut Songkram province use seablite for different types of cooking such as traditional seablite salad, seablite curry with crabs, or scalded seablite with chili paste. The edible part is the young leaves which should be scalded for about 10 to 15 min and then knocked with cold water to make them crispier (Pornpitakdamrong and Sudjaroen, 2014). In the South of India, seablite is pickled in vinegar or used for cooking as well as domestic animal food (Bandaranayke, 2002). Sudjaroen (2012) studied the nutritional values of seablite reporting that the amounts in seablite were of water, protein (3.46 ± 0.04 %w/w), fat (0.15 ± 0.01 %w/w), carbohydrate (2.18 ± 0.02 %w/w), fibers (6.21 ± 0.01 %w/w), calcium (2,471.37 ± 0.054 mg/100 g), beta-carotene (3,545.16 ± 0.093 mg/100 g). It can be seen that seablite is an interesting vegetable with high nutritional values. It has been studied that the seablite leaves can be used to prevent hepatitis (Bandaranayke, 2002; Ravikumar et al., 2011) and for antivirus. Such biological activity is related to triterpenoids and sterols. Patra et al. (2011) determined the antioxidant and antimicrobial activity of seablite's leaf and stem extracted by organic solvent (acetone, ethanol and methanol) and water. The finding was seablite extract from leaves and stems in all solvent can perform 70 to 92% of antioxidant

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from total antioxidant capacity, total phenolic content, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. From other ways to test the antioxidant of seablite, the result was also satisfying. The antimicrobial activities showed that seablite acetone extract was inhibited four types of pathogenic bacteria: *Vibrio cholera*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Escherichia coli*.

Seablite can grow naturally, so it is the low cost vegetable with high nutritional value. There should be a promotion of seablite as ready-made products for the convenience of the consumers in other regions. Moreover, it can be a new source of income of the people in the community. Pornpitakdamrong and Sudjaroen (2014) were developed dried seablite in lower scale production by using hot air blower (abc electro, Kirchheim-Teck, Germany), which can dried fresh seablite 200 to 300 g and yielded dried seablite only 30 to 50 g. On this point, seablite should be drying on larger scale and use simple technique, such as solar stove. Solar stove (size 60 x 120 cm with 4 sieves) can dry 2.5 to 3.0 kg that is10 time when compared with hot air blower and will be save energy supply, which is appropriate to people who living in rural area. The present study was aimed to produce dried seablite (*Suaeda maritima*) by solar stove, evaluate nutritive values and antioxidant activity of dried seablite during production and storage.

MATERIALS AND METHODS

Seablite collection and dried seablite production

The basis data of seablite, such as, cultivated area, last annual yield of production and local expert's interview, which were supported by Samut Songkram agricultural extension office. The seablite was harvested during December 2014 to February 2015, which scalded in boiling water added with 0.5% sugar boiling water for 5 min and after that it was soaked in cool water (Pornpitakdamrong and Sudjaroen, 2014). Scalded seabilte (2.5 to 3 kg) was blowing by electrical fan 1 to 2 h before dried by solar stove. Scalded seabilte was dried by solar stove (size 60 x 120 cm with 4 sieves) during 1 to 2 days. Internal temperature of solar stove and time of drying were recorded. The processed seablite must be dried in constant weigh prior to package in plastic bag and removed air by vacuum sealer (Karabada Sealer DZ-300A, Japan). Maximum of sunshine was optimal time for seablite drying during 10.00 a.m. to 4.00 p.m., which yielded 45 to 60°C in internal temperature of solar stove. Scalded seablite was dried within 1 to 2 days and remained 250 to 300 g of dried seablite. Preserved dried seablite in vacuumed plastic bags was100 g/each bag. Packaged dried seablite was further evaluated for nutritive values (Table 1) and biological assays, which were done in duplicate.

Evaluation of the nutritive values

Proximate analysis

The proximate analysis was carried out according to the methods to be described, or based on the Official Methods of Analysis of AOAC International, 16th ed (AOAC, 1995). The fresh samples were used for the water content determination. The remaining samples were dried at 105°C for 3 h, ground, and then stored in air-tight containers in a cool, dry place for other analyses.

Water content determination

Three to five grams of each sample was dried at 105°C for 3 h. The dried sample was then weighed. The water content was calculated as the percentage on the wet weight basis.

Determination of crude protein

Crude protein was determined by Kjeldahl method (AOAC, 1995), using Buchi Digestion Unit (B-435) and Distillation Unit (B-323) (Buchi, Switzerland). Dried sample (0.2 g) was digested with 20 ml of conc.H_2SO_4, using 3 g of the selenium and copper sulfate mixture as the catalyst. The digestion was continued for half an hour after the digestion mixture turned clear green. Then 60 ml of 32% sodium hydroxide solution was added, and the mixture was distilled for 3 min. The distillate was collected in a flask containing 60 ml of 2% (w/w) boric acid solution, with methylene blue and methyl red as the indicators. The distillate was then titrated with 0.1 N (w/w) H_2SO_4 solution; the end point was purple. Crude protein was calculated as the percentage on the wet weight basis (N × 6.25).

Determination of crude fat

One gram of the dried sample was extracted with 25 ml of petroleum ether in a Goldfisch apparatus (Labconco, U.S.A.) for 3 to 4 h. The petroleum ether extract was evaporated to dryness at 105°C. The residue was weighed and then calculated as the percentage of crude fat on the wet weight basis.

Determination of dietary fiber

Insoluble dietary fiber content was determined according to the AOAC Official Method 991.42 (AOAC, 1995). Amyloglucosidase (conc.) in the amount of 0.1 ml was used instead of 0.3 ml of the normal strength enzyme. Soluble dietary fiber content was determined according to the AOAC Official method by modified as in insoluble dietary fiber determination. The sum of both values was recorded as the total dietary fiber content of each sample.

Determination of total ash content

One gram of each sample was ignited in a muffle furnace at 525°C until ash was obtained. The residue was weighed and expressed as total ash on the wet weight basis.

Determination of carbohydrate

The carbohydrate content was obtained by difference, subtracting the water content, crude protein, crude fat, total dietary fiber, and total ash contents from 100% w/w.

Determination of β-carotene, Vitamin E and vitamin C

a) Measure β-carotene was applied from the method of Munzuroglu et al. (2003). Sample (50 g) was mashed in a homogenizer and 2 g

homogenate paste per sample was taken for extraction of β-carotene. To the above homogenates, 4 ml of ethanol were added, vortexed and the mixture centrifuged (Mistral© 2000) at 2000 rpm for 3 min at 4°C. The supernatant was also filtered through a Whatman No.1 paper, and to the filtrate 0.15 ml n-hexane was added and mixed. β-carotene was extracted twice in the hexane phase and the collected extract was dried under a stream of liquid nitrogen. Dried extract was solubilized in 0.2 ml methanol and then filtered through a 0.45 µm membrane filter before high performance liquid chromatography (HPLC) injection. Injections were made in duplicate for each sample. The quantification utilized absorption spectra of 436 nm for β-carotene. HPLC separations were accomplished at room temperature with a Perkin-Elmer liquid chromatograph system (Series 1100), consisting of a sample injection valve (Cotati 7125) with a 20 µl sample loop, an ultraviolet (UV) spectrophotometric detector (Cecil 68174), integrator (HP 3395) and a Techsphere ODS-2 packed (5 mm particle and 80 A ° pore size) column (250 × 4.6 i.d. mm) with a methanol: acetonitrile: chloroform (47:42:11, v/v) mobile phase at 1 ml/min flow rate.

b) Measure vitamin E was applied from the method of Qian et al. (1998). An initial extraction procedure was developed as follows. Sample was ground in a warring blender and screened through an 80 mesh sieve. One g of the sample was precisely weighed and transferred to a 10 ml screw-capped extraction tube. Four ml of *n*-hexane was added to the tube and the tube was flushed with a steam of N_2 to protect vitamins from air exposure before capping. The mixture was shaken on a vortex mixer for 0.5 min, rested for 5 min, and shaken another half minute. After centrifugation at 4000 rpm for 5 min, 1 ml of supernatant was transferred to a 1.5 ml vial and evaporated under nitrogen to remove the solvent. The residue was re-dissolved in 0.3 ml *n*-butanol and filtered through a 0.45 µm membrane filter before being injected into the HPLC system.

Chromatographic separations were performed on a 150 × 3.9 mm Novapak C column (Waters). Methanol was used as mobile phase at a flow-rate of 1.5 ml/min and a pressure of 1000 p.s.i. (1 p.s.i. = 6894.76 Pa) All injections were 50 µl loop injections on a M710B autosampler (Waters). A Model M510 Waters pump and a Model M490 Waters variable Wavelength UV–visible detector set at 290 nm were used. All quantitation was by peak area using a Waters M740 integrator. Based on the established chromatographic conditions, repeated injections of 0.1, 0.5, 1, 5 and 10 mg/L of the standard vitamin E was made duplicated onto the HPLC system. The retention time for vitamins E was 4.1 min. A Shimadzu MPS-2000 universal spectrophotometric scanner was used to determine the spectrograms of vitamin E in *n*-butanol.

c) Measure vitamin C was applied from the method of Sanchez-Moreno et al. (2003). Total vitamin C (ascorbic acid plus dehydroascorbic acid) were determined by HPLC. The procedure employed to determine total vitamin C was the reduction of dehydroascorbic acid to ascorbic acid, using DL-dithiothreitol as reductant reagent. 50 mg of each dried seablite was homogenized with 40 ml of an extraction solution (3% metaphosphoric acid plus 8% acetic acid).The resulting mixture was centrifuged, filtered, and adjusted to 100 ml with distilled water. Samples were filtered through a 0.45-*µ*m membrane filter, and duplicates of 20 µl for each extract were analyzed by HPLC. Results were expressed as milligrams of ascorbic acid per 100 ml. An aliquot of the mixture was taken to react with 2.0 ml of a solution 20 mg/ml DL-dithiothreitol for 2 h at room temperature and in darkness. During this time the reduction of dehydroascorbic acid to ascorbic acid has been placed. Samples were filtered through a 0.45 µm membrane filter, and duplicates of 20 µl for each extract were analyzed by HPLC. Results were expressed as milligrams of total vitamin C per 100 ml. A Hewlett-Packard model 1050 quaternary solvent delivery controller pump was used for analysis. Samples was introduced onto the column via a manual injector (Rheodyne) equipped with a sample loop (20 µl). Separation of ascorbic acid was performed by HPLC using a reversed-phase C18 Hypersil ODS (5 µm) stainless steel column (250 × 4.6 i.d. mm) (Technochroma). The solvent system used was an isocratic elution of a 0.01% solution of H_2SO_4, adjusted to pH 2.5-2.6. The flow rate was fixed at 1.0 ml/min. A Hewlett-Packard 1040A UV-visible photodiode array detector was set at 245 nm; chromatographic data and UV-visible spectra were collected, stored, and integrated using a Hewlett-Packard Chem Station and related software. Identification of the ascorbic acid was carried out by HPLC by comparing the retention time and UV-visible absorption spectrum with those of the standard previously referred to. Calibration curves were built with 10, 25, 50 and 100 mg/100 ml of ascorbic acid standard.

Determination of calcium, iron and sodium

The microwave-assisted treatment was adapted from that employed by us for the determination of the mineral profile of diets (Mir-Marqués, et al., 2015). Calcium, iron and sodium determinations were done by inductively coupled plasma optical emission spectrometry (ICP-OES) techniques. ICP -OES Optima 5300 DV inductively coupled plasma optical emission spectrometer Perkin Elmer (Norwalk, CT, USA) equipped with an auto sampler AS 93-plus, and a ultrasonic nebulizer U6000AT+ Cetac (Nebraska EEUU) were used for all mineral determination. The operating conditions of the ICP-OES equipment were as follow 15 L/min of argon plasma gas flow rate, 0.2 L/min of auxiliary gas flow rate, 0.8 L/min nebulizer gas flow rate, 1300W of radio frequency (RF) power, and 1.1 ml/min of sample flow rate. The calibration standards were prepared in 0.5% nitric acid. The calibration range for all elements was evaluated from 0.05 to 2 mg/L except calcium for which calibration curves was prepared from 2 to 10 mg/L. Ruthenium (1 mg/L) was used as internal standard and added to all samples, reagent blanks and standards.

Test of biological activities

It was used100 g of ground dried seablite for continuous extraction, then, extract with ethanol and water using Soxhlet apparatus. Finally, get the solvent evaporated through rotary evaporation apparatus under vacuum.

Total phenolic content (TPC)

Measurement using Folin-Ciocalteu reagent (Singleton et al., 1999) was done by comparing it with standard solvent, that is, gallic acid at 1 to 0.125 mg/ml concentration; then, calculating total phenolic content (TPC) of gallic acid in mg/g of the extracts.

Antioxidant activity measurement

a) DPPH radical scavenging assay to measure the decreasing light absorbance of α,α-diphenyl-β-picrylhydrzyl (DPPH) radical (Yen and Duh, 1994) using negative control by DPPH radical (6×10^{-5} M), promptly measured at 515 nm using spectrophotometer (Genesis 20, Thermo Fisher Scientific, USA). 50 µl of methanolic extract (1 to 20 mg/ml) was placed in a cuvette, and 2 ml of DPPH (6×10^{-5} M) was added and then decrease in absorbance was determined. Vitamin C (0.1 mg/ml) was used as positive controls and unit of antioxidant activity was according by mg of gallic acid equivalent, GAE.

b) ABTS cation radical scavenging assay similar to the 1st method but using2, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical instead (Re et al., 1999) and also using Trolox (water-soluble vitamin E analogue) as standard substance to create standard graph (0.5 to 5.0 mg/ml concentration). The antioxidant

Figure 1. Dried seablite processed by solar stove and preserved in vacuumed plastic bag.

activity of the dried seablite would be shown in Trolox equivalent antioxidant capacity (TEAC)/g of the dried seablite extracts.
c) Oxygen radical absorbance capacity (ORAC) to measure ability of extract to scavenge oxygen radical (Prior et al., 2003) and the florescent signal generated by fluorescene sodium salt (Sigma-aldrich, Inc.) was measured by FLUOstar OPTIMA microplate reader (BMG) on 1 h. The antioxidant activity of dried seablite would be also shown in Trolox equivalent antioxidant capacity (TEAC)/g of the dried seablite extracts.

Cytotoxic activity screening test

Test for cytotoxic activity on primate cell line (Vero cell) using green fluorescent protein (GFP)-based assay (Hunt, 1999) by ellipticine as a positive control and 0.5%DMSO as a negative control.

RESULTS

Dried seablite preparation

2.5 to 3 kg of scalded seablite was remained 250 to 300 g of dried seablite after processed by solar stove and yielded was approximately 10%. All pictures of dried seablite prepared in Figure 1.

Nutritional value of dried seablite

Nutritive values (mean ± SD) of dried seablite on beginning storage and after 3 months kept were shown in Table 1, however, there were no statistical different at $p<0.05$ when analyzed by paired *t*-test. β-carotene and vitamin E of dried seablite were vanished by scalding and drying processes on dried seablite production. Only vitamin C was still remained, however, it was decreased after 3 months storage.

Biological properties of dried seablite

As results, it was found that dried seablite extracted with water exhibited the higher TPC and antioxidant activities (except DPPH) than ethanol extract (TPC of 13.46 ± 2.35mg GAE/ g of DW, DPPH values of 15.1 ± 5.7 µmole TEAC/ g of DW, $ABTS^{+\cdot}$ values of 59.23 ± 2.4 µmole TEAC/ g of DW and ORAC values of 45.6 ± 5.6 µmole TEAC/ g of DW), which is shown in Table 2. The test about the cytotoxic activity on cell showed that hexane and ethanol extract yielded no toxic on Vero cell at 50 µg/ml of extract (Table 3). The antioxidant activity values from all methods were related to amount of total phenolic content.

DISCUSSION

Preserving of dried seablite in vacuumed plastic bags and evaluation for nutritive values, dried seablite was contained high dietary fiber (21.99 /100 g) and high

Table 1. Nutritive value of dried seablite before and after storage in 3 months*.

Nutritive value/100 g	Before	After	% Daily value**
Calories (kcal)	189.62 ±13.1	184.76 ±15.3	-
Calories from fat (kcal)	20.34 ±0.18	19.8 ±0.23	-
Total fat (g)	2.26 ±0.03	2.2 ±0.15	2
Sodium (mg)	3882.85 ±32.5	3800 ±20.6	79
Carbohydrate (g)	29.36 ±3.68	27.06 ±3.28	5
Dietary fiber (g)	21.99 ±1.44	20.5 ±1.65	44
Protein (g)	12.96 ±2.31	12.13 ±1.96	-
β-carotene	Not detected	Not detected	0
Vitamin C (mg)	0.93 ±0.001	0.3 ±0.02	0
Vitamin E (mg)	Not detected	Not detected	0
Calcium (mg)	616.02 ±15.7	610 ±18.4	30
Iron (mg)	4.41 ±0.01	4.4 ±0.01	10
Ash (g)	12.7 ±1.17	12.5 ±1.26	-

*Data are expressed as means ±SD of duplicate of assay and test for statistical different was used paired t-test ($p \leq 0.05$, 95%CI), ** % daily value is according by Thai recommends dietary allowance (Thai RDI).

calcium (616.02 mg/100 g). Dietary fiber and calcium in dried seablite were insignificant decreased (20.5 g/100 g and 610 mg/100 g, respectively) after 3 months storage. However, high amount of sodium in dried seablite should avoid intake in some risk group, such as, hypertension, cardiovascular diseases and renal diseases. Seablite was difficulty scalding for removing salty taste (Pornpitakdamrong and Sudjaroen, 2014) on summer in Thailand and unable to prepared for cooking. If seablite was prepared as dried seablite on other period, it can be useful for cooking on summer time. In addition, dried seablite is easier to transport and cook in other place where far from mangrove area that mean dried seablite can be value-added product.

These results of antioxidant tests were corresponded to the previous study (Pornpitakdamrong and Sudjaroen, 2014; Patra et al., 2011), which found water extract had higher antioxidant activities than ethanol extract. Our water extract had higher polarity, thus water extract may contain high amount of phenolic compounds that correlated to TPC as well, and however, this finding was contrasted with Patra et al. (2011) study. When comparing antioxidant activities of dried seablite to fresh seablite on previous studies (Pornpitakdamrong and Sudjaroen, 2014; Patra et al., 2011; Ravikumar et al., 2011), the antioxidant activities of dried seablite were decreased, which due to food processing, such as, scalding and drying, however, it still remained antioxidant effect in dried seablite. This results were contrasted with previous studies may depended on sample type (between dried and fresh samples), variation of plant, method of extraction, concentration, and solubility of extract and type of assay. The cytotoxic test of seablite was non-toxic (at concentration = 50 µg/ml), which was corresponded to fresh seablite on previous study (Sudjaroen, 2014) and natural antioxidants have been proved to inhibit tumor growth selectively, because of different redox status between normal cells and cancer cell (Nair et al., 2007).

Conclusion

The nutritive values of dried seablite were still remained, such as, dietary fiber and calcium and contained antioxidant substances with non-toxic property. Seablite was difficulty scalding for removing salty taste on summer in Thailand and unable to prepared for cooking. If seablite was prepared as dried seablite on other period, it can be useful for cooking on summer time, which still has preferable nutritive values and also antioxidant activities. The further study needs to test for Thai food recipes, which using dried seablite comparing with fresh seablite and also other local vegetables.

Conflict of Interest

The authors have not declared any conflict of interest.

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Table 2. Total phenolic content and antioxidant activities of water and ethanol extracts from dried seablite compared with other studies.

Extract	TPC[1]	DPPH·	ABTS+·	Other assay
Dried seablite	(mg GAE[2]/ g of DW)	(µmole TEAC[3]/ g of DW)	(µmole TEAC[3]/ g of DW)	ORAC (µmole TEAC/ g of DW)
Water	13.46 ± 2.35	15.1 ± 5.7	59.23 ± 2.4	45.6 ± 5.6
Ethanol	6.65 ± 3.24	19.8 ± 3.1	43.14 ± 3.7	32.4 ± 4.7
Fresh seablite*	(mg GAE/g of DW)	(mg GAE/g of DW)	(mg GAE/g of DW)	-
Water	0.47±2.85	14.69±0.68	61.48±8.74	-
Ethanol	6.93±2.27	20.60±0.71	44.77±4.01	-
Fresh seablite**	(%DW)	(% scavenging,100 µg/ml)	-	Nitric oxide (% scavenging, 100 µg/ml)
Water	475±0.007	~70	-	~60
Ethanol	0.77±0.004	~60	-	~70
Fresh seablite***	-	(IC_{50}, µg/ml)	(IC_{50}, µg/ml)	(IC_{50}, µg/ml)
Ethanol/Water mixture (95% V/V)		91.70 ±1.09	-	9.14±0.94

Table 3. Cytotoxic effect of dried seablite extracts to Vero cells[a, b].

Extract	Final conc. (µg/ml)	% Growth	Cytotoxicity
Water	50.0	82.60	Non-cytotoxic
	25.0	85.11	Non-cytotoxic
	12.5	95.24	Non-cytotoxic
	6.25	97.13	Non-cytotoxic
	3.125	98.11	Non-cytotoxic
	1.5625	99.49	Non-cytotoxic
	0.7813	100.00	Non-cytotoxic
Ethanol	50.0	91.35	Non-cytotoxic
	25.0	95.76	Non-cytotoxic
	12.5	97.14	Non-cytotoxic
	6.25	98.76	Non-cytotoxic
	3.125	100.00	Non-cytotoxic
	1.5625	100.00	Non-cytotoxic
	0.7813	100.00	Non-cytotoxic

[a] Positive control: Ellipticine 0.603 µg/ml, [b] Negative control: 0.5% DMSO.

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Full Length Research Paper

Regression analysis of pavement surfaces textures on noise pollution

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Noise is one of the physical environmental factors affecting human health in today's world. Pavement surface texture affects many vehicle and road characteristics; therefore, efforts are needed to develop more advanced techniques for evaluating pavement texture. The selection of an appropriate pavement as the best method to control the main cause of road noise, the sound absorption resulting from contact wheel vehicles and pavement is proposed. Finally, a case study is to determine and control the noise pollution level of skid resistance of asphalt pavements. In this study skid resistance of porous asphalt and conventional asphalt were measured. The linear regression was used for skid resistance and noise. The results have been confirmed by the nonparametric Kolmogorov-Smirnov test. Various pavement characteristics were measured and their effects on noise levels were evaluated using principal components regression, in addition to ordinary least-squares regression. This research confirmed that open graded pavements exhibit reduced tire noise compared to dense and gap graded mixes and quantified this reduction for typical mixes in Tehran.

Key words: Pavements, porous asphalt, conventional asphalt, British pendulum number, skid resistance, Kolmogorov-Smirnov test.

INTRODUCTION

Noise in cities is considered by the World Health Organization (WHO) to be the third most hazardous type of pollution after air and water pollution (WHO, 1999). One of the negative consequences of the increased traffic flow in roads and streets is increasing the noises caused by the movement of the vehicles. Consequentially, a large number of people, especially in urban areas, are exposed to dangerously high road traffic noise levels that significantly affect their health and quality of life (European Commission, 2011). Reduced noise for those living in and using the area adjacent to the roads will also yield a reduction in annoyance costs (Veisten and Akhtar, 2011).

Engine and exhaust systems' performance, cooling systems, noise caused by air collision with the body of the car, vibrations caused by the movement, the sound

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of the tires and the interactions between the tires and the pavement, creating pressure oscillations that can be easily detected by the human ear. Studies carried out by the EU Commission show that about 20% of European people endure noises more than the standard level and about 170 million square meters of cities, are annoying areas with the effects of noise pollution caused by traffic. Unit used to describe the sound pressure oscillations, as the sound level indicator, is Decibels with the symbol of dB. Because of the increase in the number of cars and industrialization, noise pollution has also increased. Noise in cities, especially along main arteries, has reached up disturbing levels. Residences far from noise sources and near silent secondary roads are currently very popular. People prefer to live in places far from noisy urban areas (Serkan et al., 2009). As, the range of the human ear listening is between zero dB (hearing threshold) to 130 dB (pain threshold). Because of the wide range of sound hearing levels and in order to provide linear diagram of these changes, the results will be used and shown with the logarithmic unit of dB (A). Pavement surface characteristics have an important role in clarifying the state of noises emission from the contact of tires and the pavement surface (Hamet et al., 1990). Previous studies in Minnesota, have shown that at speeds above 80 km/h, noise caused by contact of tires and the pavement, as the main parameter of the sounds of the vehicle in motion is considered (Hibbs and Larson, 1996).Nilsson confirmed that the noises generated by the contact of tires and the pavement surface, in the cars is the dominant noise source (National Bureau of Standards, 1970). Similar to discrete coarse aggregate, porous pavement can reduce noise generated by contact of tires and the pavement surface (National Asphalt Pavement Association, 2003). Open-graded asphalt mixes have been used in the United States for more than 50 years, primarily because they can reduce hydroplaning, water splash and spray, and therefore can reduce wet weather accident rates. Open-graded mixes have also been shown to reduce tire/pavement noise. In the last decade, open-graded mixes have been extensively used in California to obtain these benefits, while obtaining collateral benefit from their noise reducing properties (Ongel et al., 2007).

Porous asphalt tests carried out in England in 1980 led to this result that, when the coarse porous material was poured on surface, 4 to 5.5 dB noise reduction for dry conditions in comparison with conventional dense surfaces were observed (Colwill et al., 1993). In France, at the end of 1980, researchers showed that porous asphalt pavement has 1 to 6 dB more absorption ability than the compact surfaces (Berengier et al., 1990). Therefore identifying the characteristics of various types of pavement has an undeniable impact on the noise level and is very important to noise pollution control.

The sound produced by of the vehicles on the road depends on many factors such as pavement type, porosity, size and type of aggregate, features of the vehicle, and vehicle speed. Among these parameters, two factors of the speed of the vehicle and the pavement type are the most affecting ones. By improving the texture of pavement, preserving the essential factors involved in pavement design like slippery resistance etc., partly, the noise of road surface can be reduced.

Always, achieving an appropriate texture, by increasing the porosity of the pavement, in addition to better drainage, causes less vibration of tires, reduction of noise generated by air suction and better noise absorption. To achieve high porosity, it is necessary to have an open graded asphalt surface (Sandberg, 1996). According to performed investigations, the sound produced by tyre contact with the asphalt pavement is about 2 to 5 dB lower than the concrete pavement. To obtain the lowest noise level, coarse and homogenous texture must be in minimum status (Kuemmel et al., 1997). Also the rolling of the road causes the horizontal axis orientation of pebbles and thus produce less noise on be pavement surface (Sandberg, 1996). Therefore, in this research, the characteristics of pavements and the generated sound level in various porous asphalt pavement is considered. Pavement friction characteristics are provided by pavement texture qualities. Pavement texture is divided into three parts: microtexture, macrotexture and megatexture. According to the International Organization of Standardization (ISO), macrotexture is defined as deviation from the road surface from a true planar surface with the characteristic dimensions along the surface of 0.5 mm to 50 mm, corresponding to texture wavelengths with one-third-octave bands including the range of 0.63 mm to 50 mm of center wavelengths (Sandberg, 1996). In this paper the noise levels and pavement texture in two kinds of pavements has been considered and measured and the relation between them has been obtained.

METHODOLOGY

Porous asphalt pavement type is primarily based on the two main functions that reduce the noise emissions:

Rolling noise reduction: Porous pavement texture by applying the pressure of entering air to the path reduces the generated noise of crossing tires.

Sound absorption: A layer of porous asphalt, due to special aerodynamic condition, lead to reduction of the generated noise in surrounding space. Studies in the Netherlands indicate that an average reduction of 2.5 dB can be achieved using porous asphalt with normal thickness (Dutch Innovation Program on Noise Reduction, 2005; CROW, 1999, 2004). The technical specifications for porous asphalt in the case study are shown in Figure 1 and Tables 2 and 3.

This road is approximately 20 km long in west of Sari. It is a divided 4-lane road (highway) with heavy and high-volume traffic (Roudaki, 2014).

The Statistical Pass-By (SPB) method was used in the proposed project for measuring sound of vehicles in motion. In this test, sound is measured in motion. To measure sound, a vehicle crosses

(a)

(b)

(c)

Figure 1. Porous asphalt in the test area (a) Sari-Ghaemshahr road for case study (b) the sections of road for paving porous asphalt (c) Porous asphalt after paving.

at 50 km/h at a constant RPM (Round per Mile) and sensors measure the sound automatically while it crosses throughout the relevant route. The test was conducted on porous asphalt on 25 July, 2011 and Sari-Ghaemshahr highway was blocked for 10 min after making coordination with the police several times. It is only at this time that porous asphalt was used for the area of roads and because of some reasons do not use it until now. It was performed by a Peugeot model GLX405 and the sound was measured by a B&K 2236 sound meter. The device is portable and operates from a hand or a stand, aligned perpendicular to the measured surface at a distance of approximately 7.5 and 1.2 m height from the surface. Type I was measured at the distance of 7.5 m from the car and at the height of 1.2 m from maximum level of road surface. Due to the high traffic flow of the road and time limitation, the tests had to be conducted quickly without errors. The car under test should have entered the test area at 50 km/h and the accelerator should have pushed down to its maximum to cross the 300-m route of the test. The test was carried out within two areas with porous asphalt and the results shown by Table 1 were obtained.

SKID RESISTANCE TEST FOR POROUS ASPHALT

Pavement friction depends on both the microtexture of aggregates and the macrotexture of the overall pavement surface. To adequately assess the pavement friction for operational vehicles, the effects of both the microtexture and macrotexture need to be evaluated in testing and analysis.

The British Pendulum Tester (BPT), developed by the British Road Research Laboratory (RRL, now the Transport Research Laboratory, TRL), is a dynamic pendulum impact-type tester used to measure the energy loss when a rubber slider edge is propelled over a test surface. The results are reported as British Pendulum Numbers (BPNs) to emphasize that they are specific to this tester and not directly equivalent to those from other devices. The major advantage of the tester is that it can be used in the field as well as in the laboratory. However, this tester is a low-speed device (about 10 km/h [6 mph] swing speed) that measures the skid resistance related to surface microtexture rather than macrotexture since pavement friction is affected by both of these.

Table 1. The maximum noise level on porous asphalt.

Variable	Test number 1 (dB)	Test number 2 (dB)
Device 1	70/9	73/9
Device 2	71/1	71/9

Table 2. Grading of porous asphalt in the case study.

Weight percent passing each sieve	Screen size (mm)
1	
100	19
90 – 100	12.5
49 – 62	9.5
20 – 27	4.75 (#4)
9 – 20	2.36 (#8)
4 - 7	0.075 (#200)

Table 3. The technical specifications of porous asphalt.

Technical specifications	Value
Marshal of the sample or sample gyrator	50
Percentage of void	25
Percentage of drainage pitch	25
Percent by weight of the sample at 25°C	20
Indirect tensile strength ratio (AASHTO T283)	85

Table 4. British pendulum test results on porous asphalt.

Location (km)	Lane number	British pendulum number
Beginning of the road	3	69
Beginning of the road	2	64
Beginning of the road	1	63
Middle of the road	3	65
Middle of the road	2	66
Middle of the road	1	66
End of the road	3	67
End of the road	2	63
End of the road	1	61

Skid resistance is a force that acts against skidding tires on a pavement when tires rotation is hindered. Specifications of a pavement surface, including macrotexture and microtexture, are effective in slip resistance. In fact, macrotexture under wet conditions and microtexture under dry conditions control skid resistance. A British pendulum test as per ASTM E303-74 (EN 1097-8: 2009) standard was carried out under wet conditions on different points of porous asphalt to evaluate skid resistance of porous asphalt. This tester is used for examining microtexture of a pavement. It is made of a rubber pad connected to a pendulum, which oscillates on samples of a level under study. The test result is reported as British Pendulum Number (BPN).

The British pendulum tests were carried out on porous asphalt on 6 March 2011 and 7 March 2011 almost 3 months after execution. Table 4 shows the results.

CONDUCTING FIELD TESTS ON CONVENTIONAL ASPHALT IN THREE SECTIONS IN TEHRAN

The sound test was carried out on three different sections of normal asphalt in Tehran on 17 November, 2014. In each section, 5 points

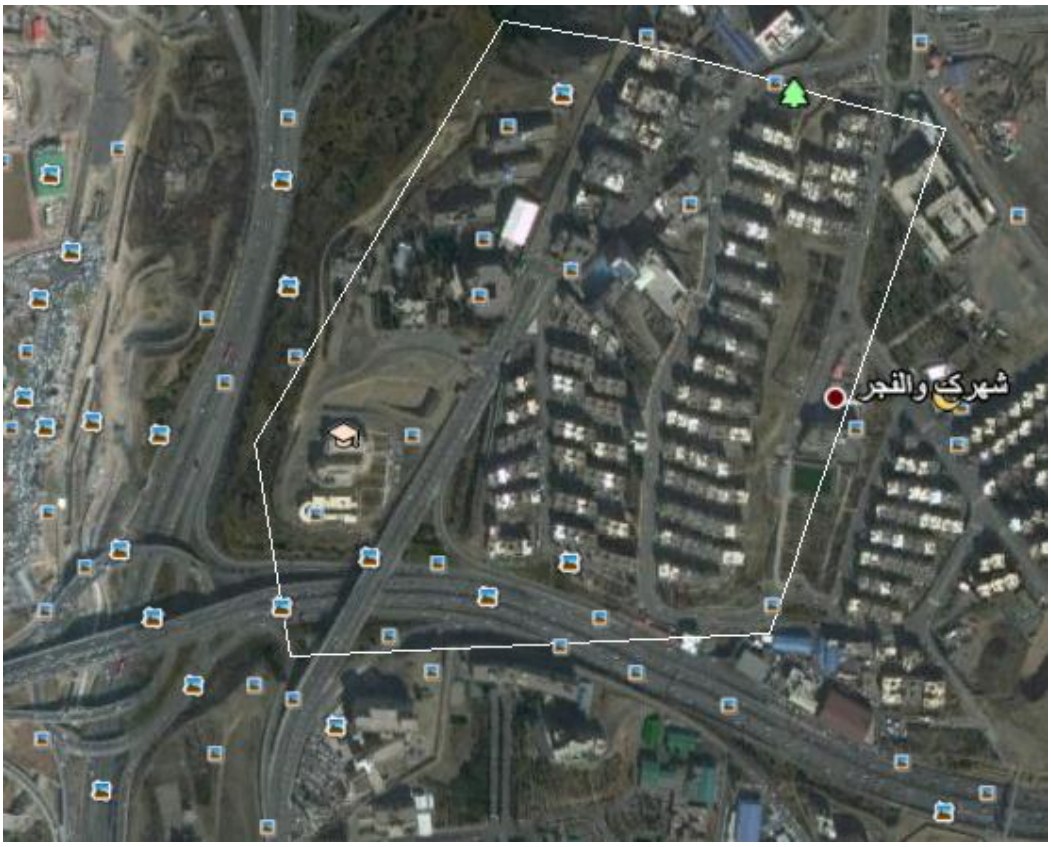


Figure 2. Case study area for field tests in Tehran.

with a distance of 200 m were selected and sound level of each point was measured. The case study area for field tests in Tehran is shown in Figure 2.

The test was conducted by a Peugeot model GLX405 and sound was measured by sound level meter TES1353. Measurements of the noise were performed using a sound level meter measuring device. The device is portable and operates from a hand or a stand, aligned perpendicular to the measured surface at a distance of approximately 7.5 and 1.2 m height from the surface. The car under test should have entered the test area at 50 km/h. Table 5 shows the results.

Skid resistance test for conventional asphalt

A British pendulum test as per ASTM E303-74 standard was carried out under wet conditions on three different points in Tehran to evaluate skid resistance. Five points with a distance of 200 m between them were selected in each section and skid resistance was measured in each point. A British pendulum test was carried out on normal asphalt in Tehran on 8 November, 2014. Table 6 shows the results. British pendulum test on conventional asphalt in Tehran is shown in Figure 3.

The steps to examine the effect of skid resistance on noise for porous asphalt of Sari-Ghaemshahr highway is as per normal asphalt.

As number of values for maximum level of sound in Sari-Ghaemshahr highway project is not sufficient, it is possible to obtain noise level – that is, y variable – by replacing BPN with x in the equation using linear regression equation for normal asphalt equation (1) obtained from SPSS output.

$$Y = 88.98 + (-0.24) \times (x)) \quad (1)$$

It should be noted that as BPN of porous asphalt consists nine values, six values were selected randomly. Table 7 shows BPNs and sound level for porous asphalt. It also shows results and analysis of SPSS for porous asphalt.

RESULTS OF STATISTICAL ANALYSIS

Here, statistical analysis for significance of the results of skid resistance test was performed. SPSS was used for examining and analyzing the relationship between BPN and noise. Simple linear regression was employed for building up relationship between data.

"*Significance Level*" (Sig) value of each parameter determines its significance, as its value for a parameter is lower than 0.05, that parameter will be significant at the confidence level of 95%. Values obtained from t-test were also reported for the parameters. In case this value for each parameter is smaller than its critical limit at that confidence level, the parameter will not be significant at that level. It should be noted that t-test is achieved by dividing the difference among mean of data into the standard deviation of their difference. Moreover, values of *Beta* column in the software output indicate effectiveness of a variable. The higher the value of a variable is, the

Table 5. The maximum noise level of conventional asphalt in Tehran.

Number	Velocity of vehicle (km/h)	Location name	Noise level (dB)
1	50	Opposite the entrance to the town Valfajr Geophysical Institute	71.5
2	50	200 m ahead across Third Street	70.3
3	50	200 m away across the street 9/7	78.6
4	50	200 m away across the street 9/3	73.2
5	50	200 m away across the street 9/1	75.7
6	50	North AmirAbad below the Hakim Highway	71.6
7	50	200 m away in front of the Atomic Energy Organization	72.5
8	50	200 m away from the National Center for Cyber Space	77.9
9	50	200 m away across the street 19	74.1
10	50	200 m away across the street 16	73.8
11	50	Dr Fatemi Street in front of the building and facilities Engineering command	75.8
12	50	200 m ahead before Sindokht Street	76.1
13	50	200 m away in front of the Iranian Fisheries Organization	79.6
14	50	200 m ahead before Etemad Zadeh Street	80.4
15	50	200 m ahead against the camp of Imam Khomeini	78.3

Table 6. British pendulum test results on conventional asphalt in Tehran.

Place of Test	Street	Lane No.	BPN
Valfajr Settlement - Opposite Institute of Geophysics	North Amir Abad Street – North of Hakim Highway	Lane 2 (Climbing Lane)	76
200 m Ahead – Opposite Street 3			74
200 m Ahead – Opposite Alley 9.7			56
200 m Ahead – Opposite Alley 9.3			68
200 m Ahead – Opposite Alley 9.1			60
North Amir Abad Street – South of Hakim Highway	North Amir Abad Street – South of Hakim Highway	Lane 2 (Climbing Lane)	59
200 m Ahead – Opposite Atomic Energy Organization			55
200 m Ahead – Opposite the National Center of Cyberspace			51
200 m Ahead – Opposite Street 19			60
200 m Ahead – Opposite Street 16			61
Fatemi Street – Opposite Buildings and Facilities Engineering Headquarter	Fatemi Street – Karegar Junction	Lane 2 (Climbing Lane)	46
200 m Ahead – Before Simindokht Street			50
200 m Ahead – Opposite Iran Fishery Organization			51
200 m Ahead – Before Etemad Zadeh Street			45
200 m Ahead – Opposite Imam Khomeini (RA) Garrison			40

higher its effectiveness is.

R^2 (R Square) expresses rate of correlation between the variables used in a regression. The closeness of the value to 1 indicates a suitable correlation among variables. The tables show the results of statistical analysis as variance analysis (ANOVA) and coefficients of the regression equation. It should be noted that the noise value mentioned by the tables is as a dependent variable and BPN was considered as independent variables.

The results of statistical analysis presented by the above table show a significant relationship between BPN and noise. In other words, *Sig* value in this mode equals 0.001 for normal asphalt and 0.000 for porous asphalt (lower than the critical value). Therefore, noise reduction for BPN is significant at the confidence level of 95%. Of course, it should be mentioned that it is not possible to accept or reject significance of a regression relation only based on a Sig value.

Research data show the effect of skid resistance on noise generation. A researcher would like to examine the question whether there is a significant relationship

Figure 3. British pendulum test on conventional asphalt in Tehran.

Table 7. The maximum noise level by placing numbers in British pendulum porous asphalt pavement in ordinary linear regression equation.

BPN for porous asphalt	Linear regression equation: Y=88.98 + (-0.24) × (x)
69	72/42
64	73/62
63	73/86
65	73/38
66	73/14
66	73/14
67	72/9
63	73/86
61	74/34
62	74/1
68	72/66
70	72/09
71	71/49
74	71/22
73	71/46

between noise generation and asphalt pavement.

To answer the fundamental question of the research, a test is conducted first as H1:ρ≠0 vs. H0: ρ=0 and then decision is made about it using the data. The following steps are taken for conducting the test of above assumption: By drawing P-P diagrams, make sure that "noise" and "skid resistance" variables are normal.

Figure 4 helps us to conclude whether these two variables are normal. (Of course, a definite conclusion on their normality is made based on a non-parametric test). Therefore, the above test should be conducted using Pearson method. The results showed a negative and significant relationship between "*noise*" and "*skid resistance*" variables. Now, we would like to discover the relationship using a regression method. Fitting stages of the regression model and its verification are as follows:

Independent variable (y) and dependent variable (x) of the research are determined first (y is noise and x is skid resistance).

Scatter diagram (which is used to know how the two variables affect each other) is as shown by Figure 5.

Table 8 shows the result of Pearson correlation test. As the significance value of the above test (Sig=0.000) is lower than 0.05, it can be concluded that the assumption H0 based on correlation (ρ≠0) between the two variables is rejected at the significance level of 0.05.

Negative value of Pearson correlation coefficient (-0.782), (-0.994) shows a negative correlation between the two variables.

Table 9 shows "percentage of data", which is explained

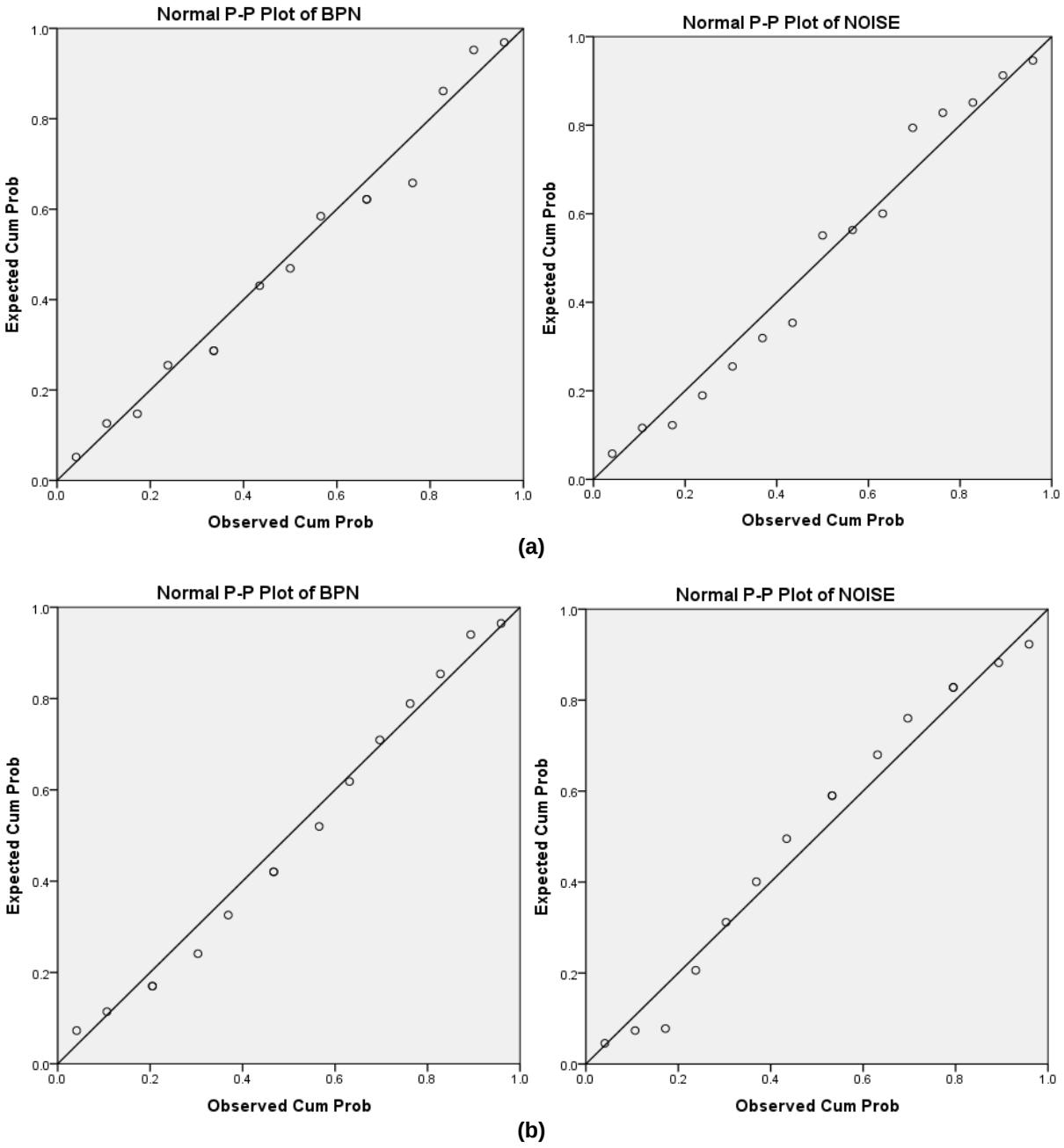


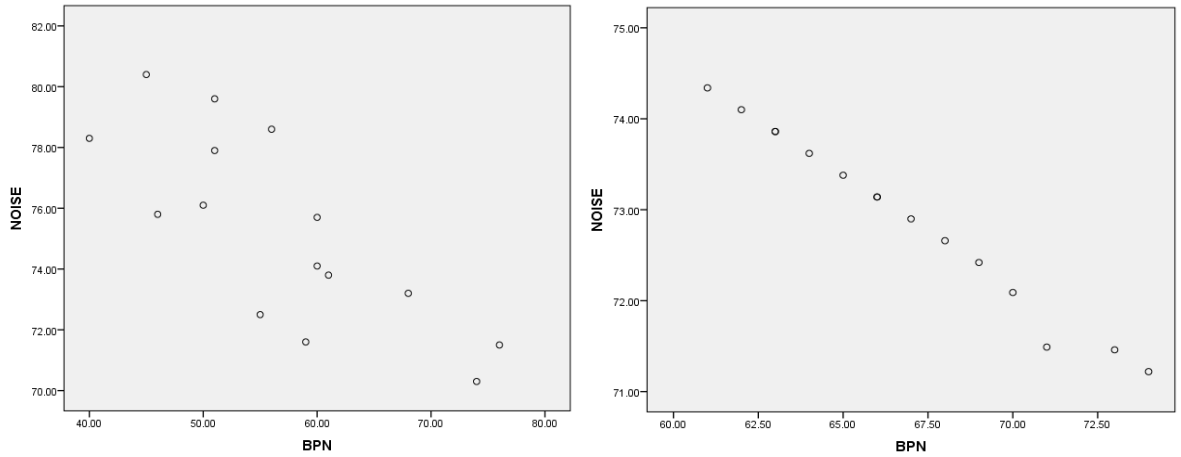
Figure 4. P-P plots (a) Conventional asphalt (b) Porous asphalt.

and described by the regression model.

It can be concluded from the adjusted correlation coefficient that 58.1 and 98.7% of the data are explained by the regression model, which is of course a favorable percentage. A test called ANOVA is used for suitability of the regression model. In fact, the test is as follows:

H0: All non-constant coefficient of a regression model equal zero vs. H1: At least one of the non-constant coefficient of the model is not zero.

The above test is known as regression model suitability. Here, it is evident that we are interested in rejecting H0 assumption. The significance value of the test (Sig=0.001, 0/000) proves suitability of the regression model at the significance level of 0.05. Table 10 shows estimate coefficients of regression model (Column B) and significance value of the test related to the coefficients. With respect to the significance value of the test concerning the constant value (Sig= 0.000), assumption of zero for the test based on the zero value is not



(a) (b)

Figure 5. The scatter diagram of skid resistance and noise level (a) Conventional asphalt (b) Porous asphalt.

Table 8. Output of the correlation.

Correlations		Conventional asphalt		Porous asphalt	
		NOISE	BPN	NOISE	BPN
Pearson Correlation	NOISE	1.000	-0.782	1.000	-0.994
	BPN	-0.782	1.000	-.994	1.000
Sig. (1-tailed)	NOISE	.	0.000	.	0.000
	BPN	0.000	.	0.000	.
N	NOISE	15	15	15	15
	BPN	15	15	15	15

Table 9. Percent values explained by the regression model.

Model	R	R^2	Adjusted R^2	Standard error of the estimate
		Model summary (Conventional asphalt)		
1	0.782[a]	0.611	0.581	2.05521
		Model summary (Porous asphalt)		
1	0.994[a]	0.988	0.987	0.11430

[a] Predictors: (Constant), BPN.

accepted at the significance level of 0.05. However, with respect to the significance value related to the independent variable coefficient (Sig=0.001, 0/000), assumption of zero for the test based on the zero value of the coefficient is accepted at the significance level of 0.05. Therefore, the regression model fitted for the data will be Equations (2) and (3).

$$y = -0.241x + \varepsilon \quad (2)$$

$$y = -0.250x + \varepsilon \quad (3)$$

Figure 6 shows a verification of the hypotheses concerning fixed value of variance and normality.

As no specific trend is seen in the remaining diagram against the fitted (predicted) values (scatter diagram), fixed amount of variance of remaining can be concluded. The above P-P diagram shows normality of remaining. As mentioned above, the result obtained from the diagram is

Table 10. Estimate coefficients of regression model.

Model		Unstandardized coefficients		Standardized coefficients	t	Significance
		B	Standard error	Beta		
Coefficients [a] (Conventional asphalt)						
1	(Constant)	88.975	3.072		28.963	0.000
	BPN	-.241	0.053	-0.782	-4.522	0.001
Coefficients [a] (Porous asphalt)						
1	(Constant)	89.598	0.513		174.716	0.000
	BPN	-0.250	0.008	-0.994	-32.592	0.000

[a] Dependent Variable: NOISE.

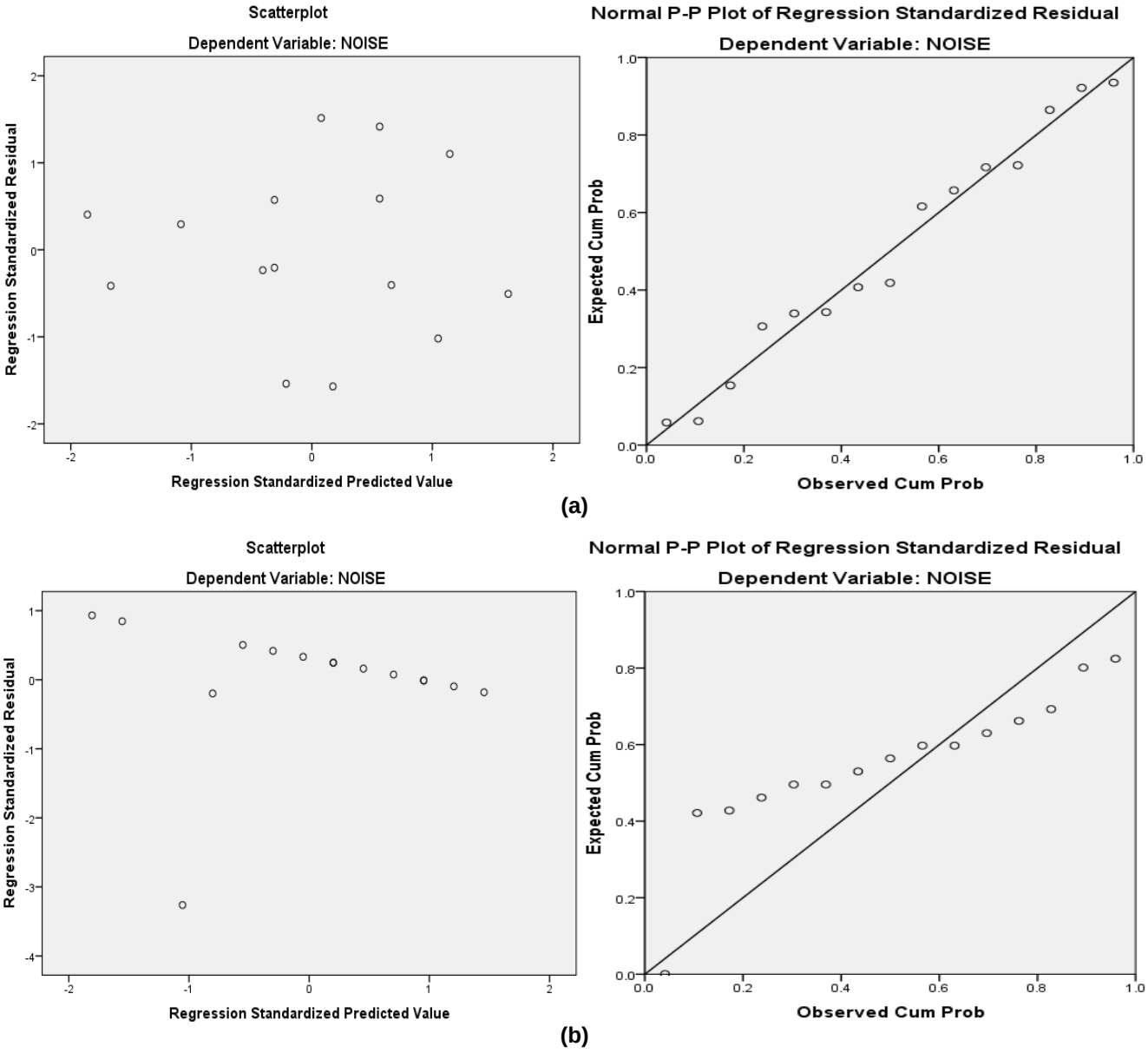


Figure 6. Scatter diagram and p-p diagram of noise (a) Conventional asphalt (b) Porous asphalt.

Table 11. Non-parametric Kolmogorov-Smirnov test.

Parameter		Standardized residual
One-Sample Kolmogorov-Smirnov Test (Conventional asphalt)		
N		15
Normal parameters[a,b]	Mean	0.0000000
	Std. Deviation	0.96362411
Most extreme differences	Absolute	0.118
	Positive	0.118
	Negative	-0.100
Kolmogorov-Smirnov Z		0.456
Asymp. Sig. (2-tailed)		0.985
One-Sample Kolmogorov-Smirnov Test (Porous asphalt)		
N		15
Normal parameters[a,b]	Mean	0.0000000
	Standard deviation	0.96362411
Most extreme differences	Absolute	0.352
	Positive	0.167
	Negative	-0.352
Kolmogorov-Smirnov Z		1.363
Asymp. Sig. (2-tailed)		0.049

[a]: Test distribution is normal. [b]: Calculated from data.

not sufficient by itself and the result should be confirmed by the nonparametric Kolmogorov-Smirnov test.

The importance of normal distribution is undeniable since it is an underlying assumption of many statistical procedures such as t-tests, linear regression analysis. When the normality assumption is violated, interpretation and inferences may not be reliable or valid. The three common procedures in assessing whether a random sample of independent observations of size n come from a population with a normal distribution are: graphical methods, numerical methods and formal normality tests. Kolmogorov- Smirnov (KS) test is one of the methods that has been used in this analysis. The normal quantile-quantile plot is the most commonly used and effective diagnostic tool for checking normality of data. The Kolmogorov–Smirnov test (hereafter the KS test) is a much used goodness-of-fit test. In particular, it is often employed to test normality, second reason for implementing normality tests is that many statistical procedures require or are optimal under the assumption of normality, and it is therefore of interest to know whether or not this assumption is fulfilled. A recent example of such a use of a normality test is given in Sanders and Lea (2005) in their study of hurricane activity.

Result in Table 11 shows conducting the non parametric Kolmogorov-Smirnov test on the reserved remaining (which are on the last column called .ZRE_1).

As the significance value (Sig=0.985) exceeds 0.05, there is no reason for rejecting assumption (H0) based on normality of remaining at the significance level of 0.05. Therefore, the normality assumption of remaining is valid.

As the significance value (Sig=0.049) do not exceed 0.05, therefore, the normality assumption of remaining is valid.

Assumption of independence of remaining and/or independence of remaining on time is examined by drawing a time series diagram of the reserved remaining as follows:

Figure 7 shows minor trend of the relationships between remaining. Of course, the trend is extremely slight and negligible. The above results can be summarized as follows:

The fitted model is on the linear relationship between *noise* and *skid resistance* variables as Equations (4) and (5).

$$y = -0.24x + \varepsilon \quad (4)$$

$$y = -0.25x + \varepsilon \quad (5)$$

Conclusion

This article mainly aimed at examining the sound generated on pavements caused by crossing vehicles and studying the effects of different parameters on it. Selecting type of pavement is the major parameter in reducing the generated sound. Some of the pavements such as porous asphalt, double-layer porous asphalt, and

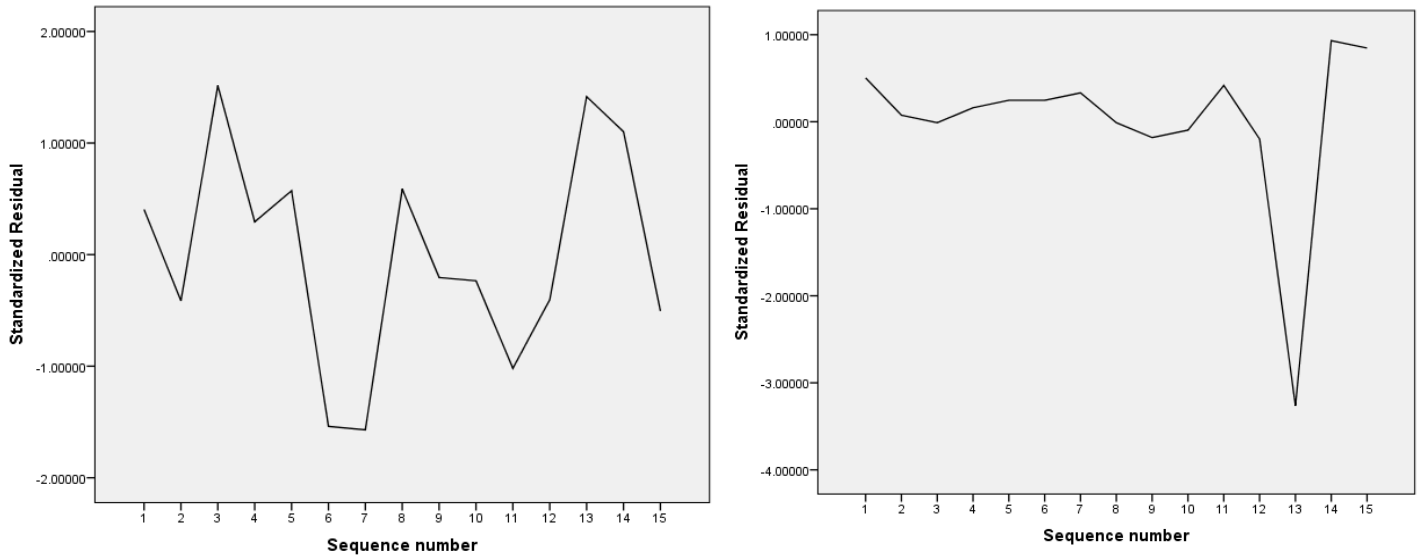


Figure 7. Time series diagram of the reserved remaining (a) Conventional asphalt (b) Porous asphalt.

stone matrix asphalt were introduced as the ones with noticeable effect on reducing sound levels. If they are selected and maintained correctly, it will be possible to reduce about 8 dB of the sound generated by crossing vehicles. As the unit used for levels of the generated sound is a logarithm-based unit, the slightest change in it can be perceived and distinguished by human ear. According to the international standard, open-graded asphalt is commonly used as the most silent pavement. Skid resistance of surface level is of the major parameters on roads noise pollution topic. It means that whenever there is not sufficient friction coefficient between contact surface of car tire and pavement, there will be more noise. In particular, humidity of pavement surface increases skidding of vehicles and the generated noise. Therefore, by examining and measuring skid resistance of pavements, the areas with unfavorable skid resistance may be identified and special measures may be taken to remove it. This research studied frictional specifications of porous-graded and normal-graded asphalt mixtures and the results are as follows:

i) A British pendulum tester can be used for examining and comparing skid resistance of asphalt surfaces.
ii) Skid resistance of porous-graded asphalt pavements is higher than the normal asphalt pavement. Here, PBN of porous-graded asphalt pavements is on average about 10 higher than the one of normal asphalt pavements.

By examination of surface of asphalt pavements, one may conclude that porous asphalt pavement, due to having macrotexture as compared with microtexture normal asphalt pavement, has a higher skid resistance on aggregates surface and causes further noise reduction. Roadways paved with open graded asphalt mixes typically generate lower traffic noise levels as compared to other types of pavements.

iii) Sound intensity measurements indicate that open-graded mixes may reduce the tire/pavement noise, compared with other asphaltic mixes. Open-graded mixes typically have higher air-void contents, permeability, and surface macrotexture than gap or dense-graded mixes.

These results indicate that the best approach for noise reduction is probably relatively thin open graded mixes with somewhat lower air void contents. These mixes will likely have greater durability due to the lower air void contents.

Tire/pavement noise is evidently affected by the characteristics of the tires and of the pavement. Only the latter factor is addressed in this paper. The physical properties of the materials that constitute the upper structural layer of the pavement play a major role in the generation of noise. Pavement mixes with higher air void contents porosity are known to reduce noise levels. There are two noise reduction mechanisms for open graded porous surfaces: noise absorption and noise propagation. The presence of air voids in the surface layer helps dissipate trapped air in the tire's tread grooves. This results in reduced air pumping, and therefore decreased noise emissions.

Conflict of Interest

The authors have not declared any conflict of interest.

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Agriculture germplasm resources: A tool of conserving diversity

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Three major physical resources in the world comprise land, water and the biological diversity. Agricultural biodiversity is an important component of biodiversity, which has a more direct link to the well being and livelihood of mankind than other forms of biodiversity. In fact, it is one of our most fundamental and essential resources, one that has enabled farming systems to evolve since the birth of agriculture about 10,000 years ago. Food plant and animal species have been collected, used, domesticated and improved through traditional systems of selection over many generations. The resulting diversity of genetic resources developed by early farmers now forms the basis on which modern high yielding and disease resistant varieties have been produced to feed the growing human population, expected to reach 9.1 billion by 2050. According to the Convention on Biological Diversity (CBD), "agricultural biodiversity includes all components of biological diversity of relevance to food and agriculture, and all components of biological biodiversity that constitute agro-ecosystems: the variety and variability of animals, plants and micro-organisms, at the genetic, species and ecosystem levels, which are necessary to sustain key functions of the agricultural ecosystem, its structure and processes". The effective conservation and use of agricultural biodiversity is very important in ensuring sustainable increases in the productivity and production of healthy food by and for mankind as well as contributing to increased resilience of agricultural ecosystems.

Key words: Agricultural biodiversity, ecosystem, Convention on Biological Diversity (CBD), domestication, human population, variability.

INTRODUCTION

There are many threats or drivers of changes on biodiversity that have been recognized and intensified in recent years (Millennium Ecosystem Assessment, 2005). With regard to agriculture the most important ones include changes in land use, replacement of traditional varieties by modern cultivars, agricultural intensification, increased population, poverty, land degradation and environmental change (including climate change) (FAO, 2010). It is predicted that climate change will have a significant impact on agriculture with temperatures

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rising on average by 2 to 4°C over the next 50 years, causing significant changes in regional and seasonal patterns of precipitation (Burke et al., 2014). Climate change will also impact agricultural biodiversity in a major way. Model projections carried out by Lane and Jarvis (2007) based on global distribution of suitable cultivated areas of 43 crops, highlight that more than 50% may decrease in extent. Evidence based on bioclimatic modelling suggests that climate change could cause a marked contraction in the distribution ranges of CWR. In the case of wild populations of peanut (*Arachis* spp.), potato (*Solanum* spp.) and cowpea (*Vigna* spp.), studies suggest that 16 to 22% of these species may go extinct by 2055, with most species possibly losing 50% of their range size (Jarvis et al., 2008). These threats or drivers of change are leading to large scale degradation and loss of agricultural biodiversity and consequently its genetic variability (Millennium Ecosystem Assessment, 2005; van de Wouw et al., 2009). Information regarding the threat and rate of genetic erosion among various components of agricultural biodiversity is important, yet very little work has been carried out to quantify the magnitude of any trends. The availability of large gene pools, including CWR, is becoming even more important as farmers will need to adapt to changing conditions that result from these pressures. It is likely that many of the genetic traits which will be necessary to adapt our crops to changing climate will be found in CWR. Hence, it is widely urged that such strategies be adopted which may be used to get maximum crop stand and economic returns from adverse environments. Major strategies which may be used to overcome the adverse effects of such stressful environments may include screening and selection of well adopted existing germplasm of potential crops (Ahmad et al., 2014).

There are two main strategies for conserving agricultural biodiversity, namely *ex situ* and *in situ* conservation, both of which are equally important and should be regarded as complementary (Thormann et al., 2006; Engelmann and Engels, 2002). *Ex situ* conservation is the conservation of components of biodiversity outside their natural habitats. It is generally used to safeguard populations that are at present or potentially under threat and need to be collected and conserved in genebanks in the form of seeds, live plants, tissues, cells and/or DNA materials. Article 2 of the CBD defines *in situ* conservation as “the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties” (UNCED, 1992). It thus refers to the maintenance of a species in its natural habitat. This can be either on farm, requiring the maintenance of the agro-ecosystem along with the cultivation and selection processes on local varieties and landraces, or in the wild, which involves the maintenance of the ecological functions that allow species to evolve under natural conditions.

STATUS AND TRENDS OF AGRICULTURAL BIODIVERSITY

Little is known about the global status of agricultural biodiversity. Although the CBD recognize genetic diversity as one of the fundamental levels of biodiversity, actions to protect genetic diversity are lacking (Laikre et al., 2010). Policy makers and scientists require a better understanding of how the intraspecific diversity is changing over time and space in order to make informed decisions for their conservation. However, there is no routine global scale monitoring of genetic diversity over time (Frankham, 2010; Laikre et al., 2010), except for a few target species at national level (Laikre et al., 2008). A major challenge remains to develop simple inexpensive means to monitor genetic diversity at a global scale (Frankham, 2010). Several efforts under the 2010 Biodiversity Indicators Partnership (http://www.twentyten.net) have been made to identify indicators useful to detect changes in species and ecosystem diversity, but there are only two initiatives that are explicitly working on developing indicators that deals with genetic variation for agricultural biodiversity (Laikre, 2010; Walpole et al., 2009).

The only authoritative account of agricultural biodiversity status at the global level is represented by the First and Second reports on the *State of the World’s Plant Genetic Resources for Food and Agriculture* published by the Food and Agriculture Organization of the United Nations (FAO, 1998, 2010). The Second Report mention that there are about 7.4 million accessions conserved in over 1750 gene banks around the world in either seed banks, field collections, and *in vitro* and cryopreservation conditions (FAO, 2010). This represents an increase of more than 1.4 million accessions added to *ex situ* collection since publication of the first report on the *State of the World’s Plant Genetic Resources for Food and Agriculture*. Although reportedly over-represented, a large part of the genetic diversity of major food crops is stored in *ex situ* collections. The exact proportion is still uncertain, but estimates suggest that more than 70% of the genetic diversity of some 200 to 300 crops is already conserved in gene banks (SBSTTA, 2010). In addition there are over 2,500 botanic gardens maintaining samples of some 80,000 plant species (FAO, 2010). However, regeneration of gene bank accessions remains a major problem, threatening collections (FAO, 1998). In the past decade there have been significant advances made in regenerating collections at risk, in part due to efforts made by the Global Crop Diversity Trust (CGDT) in supporting regeneration programmes of globally important priority gene bank collections for 22 priority crops for which crop specific regeneration guidelines

have recently been produced (Dulloo et al., 2013). Another major achievement has been the creation of the Svalbard Global Seed Vault (SGSV) in 2008, established to serve as the ultimate safety net for seeds samples from the world's most important collections (GCDT, 2010).

Great efforts for the conservation of many CWRs and wild species have been made by the Millennium Seedbank (MSB) at Wakehurst Place, Royal Botanic Gardens, Kew, UK which aims to house up to 10% of the world's seed-bearing flora, principally from arid zones by 2010. Genetic erosion has also been prevented by the significant amount of crop genetic diversity in the form of traditional varieties and neglected and underutilized species (NUS) that continues to be maintained on-farm. Yet, in spite of these advances, important reservoirs of adaptive variation such as CWR, landraces and NUS, which are increasingly recognized by the global scientific community as key resources for the maintenance of agro biodiversity, remain under-represented (FAO, 2010). CWR in particular, which have avoided the genetic bottleneck of domestication, contain greater genetic variation than their cultivated relatives and represent an important reservoir of genetic resources for breeders (Maxted and Kell, 2009). Yet to retain the genetic characteristics that make them so valuable for crop improvement, it is now widely recognized that populations of CWR are best conserved *in situ*, in their wild habitats, where they can continue to adapt and evolve along with their natural surroundings, thus ensuring new variation is generated in the gene pool and the continued supply of the novel genetic material critical for future crop improvement. The underpinning of the conservation strategy of most countries is a protected areas system and this is reflected in the CBD, where the main thrust of biodiversity conservation is *in situ*, through the development of such protected systems. Populations of many CWR occur in existing protected areas, but this alone does not in many cases represent effective *in situ* conservation without some degree of management or intervention targeted at the populations of the particular target species, particularly if the species is threatened. Despite protected areas being in existence for many years we still have not been able to undertake significant actions to conserve the CWR they contain, except a few cases.

Despite this, the *in situ* conservation of CWR has gained increasing attention in many countries, as demonstrated by their inclusion in the many national reports drafted for the Second report on the *State of the World's Plant Genetic Resources for Food and Agriculture* (FAO, 2010). Unfortunately, little quantitative data were provided by countries on the changing status of CWR, but several reports indicated that specific measures had been taken to promote their conservation. The Second report also mentions that surveys and inventories of CWR were carried out in at least 28 countries and many new priority sites for conserving CWR *in situ* have been identified over the last decade. There is also evidence that public awareness of the importance of CWR, and neglected and under-utilized species such as traditional vegetables and fruits, is growing both in developing and developed countries (FAO, 2010). This has been furthered by a number of global initiatives aimed at conserving CWR, such as the proposed establishment of a global network for the *in situ* conservation of CWR (Maxted and Kell, 2009), and more concretely by the creation of web-based international platforms for the exchange of CWR information and data. These include the European platform "An Integrated European *In Situ* Management Work Plan: Implementing Genetic Reserves and On Farm Concepts" (AEGRO) and the CWR Global Portal, developed as part of the UNEP/GEF Crop Wild Relative Project, that provides access to CWR information and data at the global level (Thormann et al., 2012). The significant increase in number of scientific articles published on CWR and on specific actions targeting their conservation is also a testimony to the renewed interest in CWR, however, to the best of our knowledge few of the recommendations have been implemented, largely due to a lack of funds and capacity.

Over the last decade, the number and coverage of protected areas has increased by approximately 30% (United Nations, 2010), yet limited efforts have been made to target CWR, whose conservation remains unplanned and largely an indirect effect of protecting flagship species or threatened habitats. For example, despite the increase in isolated activities targeting CWR conservation, the formal recognition and/or the adoption of appropriate management regimes to protect CWR are largely lacking. Furthermore, considering that national parks and other conservation areas cover only 12 to 13% of the earth's surface, it is clear that these areas alone will not be able to ensure the continued existence of CWR species, the majority of which occur in marginal lands outside protected areas, where no form of legal protection is offered. If protected areas are to ensure the long-term survival of CWR they will need to become more flexible in size and scale and a connected network of habitats will need to be established to allow species to migrate and adjust their ranges in response to global change and anthropogenic disturbances, along with the development of effective management strategies targeting their conservation (that is, off-reserve management). The success of this strategy will depend largely on promoting more biodiversity-sensitive management of ecosystems outside protected areas, and successfully engaging private landowners and local communities living around protected areas in the conservation process. Finally, more effective policies, legislation and regulations that take into account the impacts of global changes on future species distribution and that govern the *in situ* conservation of CWR, both inside and outside of protected areas, are needed, along

with closer collaboration and coordination between the agriculture and environment sectors.

Formidability of genetic resources

Wild plants have often played an important role in many diets due to their higher nutritional value than cultivated species. These are, at the same time, hardy and resilient. Crop varieties are improved by the suitable recombination of genes from the wild, made more productive, resistance to biotic stresses, tolerant to abiotic stresses, and better nutritional and keeping quality. Such characteristics, needed to improve crop varieties, may be found in a range of cultivated as well as wild plants. This broad variability provides essential link in the food chain, which, in turn, provides the basic for world food and nutritional security. Plant genetic resources essentially constitute the prime components of the food chain ever since the dawn of agriculture. In the history of some 12,000 years, nearly 30,000 edible plant species have been utilized as a source of food. However, merely a hundred odd plant species out of these have been propagated to provide about 90% of the world food and, further, only three species among these, namely, rice, wheat and maize produced the two-third. An assessment of the contribution of different plant sources towards the dietary energy supply at the global level shows predominance of only two crops, that is, rice (26%) and wheat (23%) (FAO, 1996). The search for new diversity is, therefore, important.

In the developing and the economically weaker parts of the world, the discovery of wild species for food may have coincided with the hunger season, such as, those preceding the crop harvest particularly when drought or flood situations occurred. Mother Nature provided food for people at such junctures when they badly needed it and the resultant discovery of plant species or their diversity became the automatic human choices for further propagation. Even today, though agriculture has advanced so much, humans still gather many wild and semi-wild plants or plant parts like fruit, leaf root, seed, nut, wood etc. for use. About 80,000 species of plants have been used to meet the routine needs by the human beings. Of these, 30,000 species so far have been identified as edible and about 7,000 species have been cultivated and/or collected for food at one time or the other. Presently only 20 to 30 crops, such as cereals (wheat, rice, maize, millets, sorghum), root/tuber crops (potato, sweet potato, cassava), legumes (pea, beans, peanut, soybean) and sugarcane, sugar beet, coconut and banana are mainly used to feed the world (NAS, 1975).

Synoptic view of plant diversity

There are 425,000 species in living plants in the plant kingdom from unicellular algae to the highly evolved flowering plants. The flowering plants are a diverse group which are seed producing plants that have evolved in synchronization with the evolution of insects which help the plants in cross-pollination assuring heterozygous population. Hence, this group of flowering plants (about 250,000 species) have developed great plasticity for adaptation for different climatic regimes. They consist of a variety of life forms from the minute Wolffia (1 mm long) to the largest Eucalyptus regnant growing to height of 100 m. This spectrum of flowering plants includes humble herbaceous species, beautiful orchids, parasitic *Rafflesia arnoldii* having largest flowers (1 m across), plants of medicinal value, trees of timber importance, food plants, fodder species, gums and resin producing plants. The 250,000 flowering plant species are packed in about 17,000 genera and 300 to 400 families. Some of the economically important families which hold life supporting food sustenance species are Gramineae, Legumonosae, Criciferae, Cucurbitaceae, Rosaceae, Brassicaceae and Rutaceae. The drug yielding families cover a spectrum of alkaloids producing crops such as, Apocynaceae, Papavaraceae, Asteraceae, Cannabinaceae, Piperaceae, Zingiberaceae and Rubiaceae. Gums and resins occur in several families as Eurphorbiceae, Dipterocarpaceae, Mimosaceae and Sapotaceae. The families vary in size from monotypic ones to large families having 25,000 to 35,000 species. The family Orchidaceae has about 25,000 to 35,000 species, Compositae has about 20,000 species, Legumionceae has about 14,00 species and Gramineae has about 8,000 species, while there are about 35 families which has only one species.

Plant diversity in India

Indian subcontinent has a rich and varied heritage of biodiversity, encompassing a wide spectrum of habitats from tropical rainforests to alpine vegetation and from temperate forests to coastal wetlands. It is one of the eight centres of origin (Vavilov, 1951) and is one of the 12 mega gene centres of the world; possess 11.9% of world's flora. About 33% of the country's recorded flora are endemic to the region and are concentrated mainly in the North-East, Western Ghats, North-West Himalays and Andaman and Nicobar islands, nurish one third of the human population on this earth (Damania, 2002; Mayres et al., 2000) have brought out an updated list of 25 global hotspots of diversity out of which 8 hotspots are in figured in India. Indian sub-continent is a centre of domestication and diversification of several economically useful wild plant species comprising about 3,000 plants of edible value, 4,000 species having known reputed medicinal value, 700 plants of traditional and social significance, 500 fibre yielding species, 400 fodder species, 40 species having insectivorous uses, 300 gum and dye yielding plants, 100 aromatic and essential oil yielding

species (MoEF, 1994; Chowdary and Murti, 2000). Indian diversity comprises of 49,219 higher plant species, out of which, 5,725 are endemic and belonging to 141 genera under 47 families. Of these endemic species, 3,500 are found in Himalayas and adjoining regions and 1,600 in the Western Ghats alone.

India is a homeland of 167 cultivated species and 329 wild relatives of crop plants (Arora, 1991). It has around 30,000 to 50,000 landraces of rice, wheat, Pigeonpea, mango, turmeric, ginger, sugarcane, etc. and ranks 7th in terms of contribution to world agriculture. Further, around 1,000 wild edible plant species are exploited by native tribes. These include 145 species of roots and tubers, 521 of leafy vegetables/greens, 101 of buds and flowers, 647 of fruits and 118 of seeds and nuts (Arora and Pandey, 1996). In addition, nearly 9,500 plant species of ethano-botanical uses are reported from the country of which around 7,500 are of ethano-medical importance and 3,900 are multipurpose edible species.

The endemic plant wealth of the country has also been supplemented with the species/forms that had been introduced from abroad. These species got naturalized over time and have undergone the process of domestication on being isolated climatically and spatially. Prominent among these aer apple, pear, peach, apricot, grape, almond, date palm, maize, potato, sweet potato, tomato, bean, onion, garlic, chilli, lentil, clove, coriander, cumin, fennel, coffee, cocoa, cashew nut, litchi, cinchona, strawberry, blueberry, tea, rubber and pineapple.

Biodiversity in Jammu and Kashmir

The State of Jammu and Kashmir has been regarded as heaven on earth, and is also called the bio-mass state of India. This area, located in the far north of the Indian republic, is a mountainous zone in the north-west Himalayas that shares international boundaries with Pakistan in the west, Chinese autonomous region of Xinjiang in the north and Tibet in the north-east. The North-western-Himalayan region being the rich repository of biological heritage, particularly in respect of agri-horticultural crops and was recognized that collection and maintenance of germplasm is essential to provide genetic diversity within a crop and to reduce chances of genetic vulnerability. Exploration and collection of native biodiversity, particularly in agri-horticultural crops of the region, including the wild relatives, rare/endangered plants together with the documentation of related ethano-botanic information for exercising to concomitant with regeneration and preliminary evaluation of the collected genetic resources to ensure their long term conservation as well as use in crop breeding programmes recognizing the fact that improvement and sustenance of cultivated crop species requires variability.

A total of 1911 germplasm accessions comprising local cultivars that were in cultivation before introduction of improved cultivars , old varieties, land races, wild crop relatives and under-utilized crops of agri-horticultural significance were collected in respect of various field, vegetable , and horticultural crops as well as medicinal and aromatic plants. The collected biodiversity included 742 accessions in cereals, 38 in pseudo cereals, 28 in millets,71 in oilseeds, 358 in pulses, 377 in vegetable crops, 21 in spices and condiments, 13 in fodder crops, 204 in medicinal and aromatic plants, 55 in fruits crops and 4 in others. The collection of agro-biodiversity in different crops has not only helped in ensuring their conservation on a long term basis but their use may also increase productivity, food security and economic returns. The valuable biological resources will make the farming systems more stable and sustainable. By establishing suitable linkages with user scientists in the university and sister institutions in the region a total of 382 accessions in cereals, 135 pulses, 78 vegetables, 26 horticultural crops, 110 in medicinal and aromatic plants were made available for use in respective crop improvement programmes. Their eventual use in the development of varieties with high yield potential and improved quality characteristics may diversify production and income opportunities for the end user.

Conservation of germplasm

Global concern about loss of valuable genetic resources prompted international action. Programs for conservation of plant genetic resources for food and agriculture were thus initiated and gene banks established in many countries. The main objective was to collect and maintain the genetic diversity in order to ensure its continued availability to meet the needs of different users. The concept of germplasm conservation demands that collection methods initially capture maximum variation and subsequently, conservation and regeneration techniques minimize losses through time. To this effect, plant genetic resources (PGR) conservation activities comprise collecting, conservation and management, identification of potentially valuable material by characterization, and evaluation for subsequent use.

There are two approaches for conservation of plant genetic resources, namely *in situ* and *ex situ*. *In situ* conservation involves maintaining genetic resources in the natural habitats where they occur, whether as wild and uncultivated plant communities or crop cultivars in farmers' fields as components of the traditional agricultural systems. *Ex situ* conservation on the other hand, involves conservation outside the native habitat and is generally used to safeguard populations in danger of destruction, replacement or deterioration. Approaches to *ex situ* conservation include methods like seed storage, field gene banks and botanical gardens. DNA and pollen storage also contribute indirectly to *ex situ* conservation of PGR.

Ex situ conservation approach

Ex situ conservation refers to the conservation of germplasm away from its natural habitat. This complementary approach for conservation had begun on a wide scale about three decades ago and is now practised, to some extent, in almost all countries as a means to conserve crop species diversity for posterity. This strategy is particularly important for crop gene pools, and can be achieved by propagating/ maintaining the plants in genetic resource centre, botanical gardens, tissue culture repositories or in seed gene banks. The Second Report mention that there are about 7.4 million accessions conserved in over 1750 genebanks around the world in either seed banks, field collections, and *in vitro* and cryopreservation conditions, (FAO, 2010).

Various approaches are employed for the *ex situ* conservation depending upon the mode of reproduction and nature of plants to be conserved. *Ex situ* conservation approach generally comprises the following methods: seed storage, field gene banks, *in vitro* storage, pollen storage, DNA storage and botanical gardens.

Seed storage

In the past, many collections were maintained without the help of storage facilities which would affect the viability of seeds. Due to this, the conserved accessions had to be regenerated very frequently leading to loss of genetic diversity in gene banks (Frankel and Hawkes, 1975). In maintaining genetic purity of the conserved accessions, problems arise due to differential survival in storage, selection during regeneration, out-crossing with other entries and genetic drift (Allard, 1970). Good storage conditions coupled with proper grow-outs are expected to reduce the effects of such problems (Rao, 1980).

Storing orthodox seeds at low moisture content and at subzero temperature is the most convenient and widely used method of genetic conservation. Orthodox seeds are the seeds which can withstand dehydration without damage. This type of seeds can be stored in the dry state on long term basis (indefinite period) which can be prolonged by decreasing their moisture content and storage temperature (at sub zero temperature).

The number of seed storage facilities has increased dramatically over the last two decades. Today, according to the WIEW – World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture – databases of the FAO, there are 1320 national, regional and international germplasm collections in the seed form, 397 of which are maintained under long- or medium-term storage conditions. Over 6.1 million accessions have been conserved as seeds.

As opposed to common orthodox seeds, there are a number of species whose seeds are unable to withstand desiccation, that is, cannot be dried to low levels for optimum storage. Such seeds are referred to as 'recalcitrant' seeds (Roberts and King, 1986). Mainly these seeds originate from the plants grown in tropical and sub-tropical regions. These seeds can be stored for short duration (up to several months) by imbibed storage (at higher levels of seed moisture/hydrated state) and relatively warm conditions (well above zero temperatures) because they are often chilling sensitive. e.g., rubber, cocoa, coconut. Seeds such as oil palm and coffee, showing intermediate storage behaviour (Ellis et al., 1990, 1991) were grouped as recalcitrant until recently. Careful adjustment of the desiccation level and storage conditions allowed their storage for increased period (1 to 2 years).

Very low temperature storage using liquid nitrogen, called cryo-preservation, appears to be promising, with a more extended life span, described as long-term storage (-20°C). Another area in which considerable work is required is on storage of ultra dry seeds (dried to seed moisture content of 2 to 5%) at room temperature conditions and in hermetically sealed containers (Zhou et al., 1995). However, more research will be necessary before ultra dry seed technology can be adopted (Zheng et al., 1998). Prior to embarking on any seed conservation programme, a decision is to be made on how long it will be necessary to maintain the germination capacity of the seed lots, because longer storage requires more exacting storage conditions. This shall be determined by the objective of the conservation which could be research, introduction, breeding, etc.

Field gene bank conservation

Many important varieties of field, horticultural and forestry species are either difficult or impossible to conserve as seeds (that is, no seeds are formed or if formed, the seeds are recalcitrant) or reproduce vegetatively. Hence they are conserved in field gene banks (FGB). FGBs provide easy and ready access to conserved material for research as well as for use. It is one of the options of a complementary strategy for the conservation of germplasm of many plant species.

The conservation of germplasm in field gene bank involves the collecting of materials and planting in the orchard or field in another location. Field gene bank has traditionally been used for perennial plants, including:

i) Species producing recalcitrant seeds;
ii) Species producing little or no seeds or sterile seeds;
iii) Species that are preferably stored as clonal material;
iv) Species that have a long life cycle to generate breeding and/or planting material.

Field gene banks are commonly used for such species as cocoa, rubber, coconut, coffee, sugarcane, banana, cassava, sweet potato, yam, tropical and temperate

fruits, vegetatively propagated crops (e.g. wild onion and garlic) and forage grasses (e.g. sterile hybrids or shy seed producers). This is the traditional method of conservation to keep the germplasm in plantations as mature trees. It provides mature material for vegetative propagation, hybridization and characterization. The site for a field gene bank should have a suitable climate and soil for the species and should have an adequate water supply. The site should be chosen in a location with little or no threat of pests, diseases, bush fire and vandalism.To avoid loss of vigour as well as to prevent the incidences of attack by pests the plants have to be replanted routinely, and this adds to the cost further.

Botanical gardens

There are about 2500 botanic gardens and arboreta worldwide. It is estimated that these gardens maintaining samples of some 80,000 plant of threatened species in botanical gardens and arboreta. The objectives of most of the gardens include:

(a) Maintaining essential ecological processes and life support systems,
(b) Preserving genetic diversity, and
(c) Ensuring sustainable utilization of species and ecosystem.

However, the botanical gardens may play a limited role in the context of conservation and propagation and probably a greater role in public awareness and education. Botanical gardens may mainly be used to display a great number of different and exotic species. As the number that can be maintained in this manner is limited, it cannot reflect or conserve genetic diversity. There is a possibility that a few well-managed gardens can emphasise on conservation of certain groups of species as living collections (that is, field gene banks). Often botanical gardens focus their conservation efforts on wild, ornamental, rare and endangered species. Indeed botanical garden conservation could be considered as field gene bank and/or seed gene bank, depending on the conservation method being used. The living plant collections in botanic gardens and arboreta may be considered as field collections, but the original purpose of the gardens and arboreta is not for germplasm conservation. Most of the germplasm conserved in botanical gardens do not belong to the PGRFA.

***In vitro* storage**

Research on finding solutions to better conserve these difficult-to-store seeds has focused on the use of biotechnology (Engelmann and Engels, 2002). *In vitro* slow-growth conservation methods, involving culturing different parts of the plant (meristem, tissues, cells) into pathogen-free sterile culture in a synthetic medium with growth retardants have been cited as good ways of complementing and providing backup to field collections. It has long been known that *in vitro* slow growth method suffers high risks of somaclonal variation (Withers, 1993) and also from the need to develop individual maintenance protocols for the majority of species (Thormann et al., 2006).

Slow growth

Slow growth procedures allow clonal plant material to be held for 1 to 15 years under tissue culture conditions with periodic sub-culturing, depending on species. There are several methods by which slow growth can be maintained. In most cases, a low temperature often in combination with low light intensity or even darkness is used to limit growth. Temperatures in the range of 0 to 5ºC are employed with cold tolerant species, but for tropical species which are generally sensitive to cold, temperatures between 15º and 20ºC are used. It is also possible to limit growth by modifying the culture medium, mainly by reducing the sugar and/or mineral elements concentration and reduction of oxygen level available to cultures by covering explants with a layer of liquid medium or mineral oil (Withers and Engelmann, 1993). Although slow growth procedures have been developed for a wide range of species, they are routinely used for conservation of genetic resources of only a few species including *Musa* spp., potato, sweet potato, cassava, yam, *Allium* spp. and temperate tree species. About 37,600 accessions are reportedly conserved by *in vitro* techniques in gene banks, worldwide (FAO, 1996).

Cryopreservation

Cryopreservation, the process in which living tissues are conserved at very low temperatures (-196°C) in liquid nitrogen (LN) or in vapour phase (-150°C) to arrest mitotic and metabolic activities, provides a more promising option (Thormann et al., 2006). Significant progress has been made in cryopreservation research over the past twenty years and much of that research has been focusing on understanding the desiccation sensitivity of recalcitrant seeds and on the underlying mechanism of desiccation tolerance (Engelmann and Panis, 2009). The techniques for cryopreservation currently in use are quite varied and include the older classical techniques based on freeze-induced dehydration of cells as well as newer techniques based on vitrification (Engelmann, 2000). In classical techniques, tissues are cooled slowly at a controlled rate (usually 0.1-4°C/min) down to about -40ºC, followed by rapid immersion of samples in liquid nitrogen. Slow

freezing is carried out using a programmable freezing apparatus. Cryoprotectants are added to the freezing mixtures to maintain membrane integrity and increase osmotic potential of the external medium. Classical cryopreservation procedures have been successfully applied to undifferentiated culture systems such as cell suspensions and calluses (Kartha and Engelmann, 1994). However, in case of differentiated structures, they have been employed for storage of apices or embryonic axes of only cold-tolerant species (Reed and Chang, 1997), and their utilization for tropical species has been limited (Escobar et al., 1997). Vitrification-based procedures involve removal of most or all free able water by physical or osmotic dehydration of explants, followed by ultra-rapid freezing which results in vitrification of intracellular solutes, that is, formation of an amorphous glassy structure without occurrence of ice crystals which are detrimental to cellular structural integrity. These techniques are more appropriate for complex organs like embryos and shoot apices; they are also less complex and do not require a programmable freezer, hence are suited for use in any laboratory with basic facilities for tissue culture.

DNA storage

With the rapid development in the field of molecular genetics and genomics, DNA material is becoming more and more in demand for molecular studies and is one of the most requested materials from gene banks (Andersson, 2006). The establishing of a DNA storage facility as a complementary "back-up" to traditional *ex situ* collections has been suggested (Dulloo et al., 2013), but little effort has been made to collect and conserve DNA as a genetic resource. Some efforts have been made to establish DNA banks for endangered animals (Ryder et al., 2000) and a few plant DNA banks including Missouri Botanic Garden, Royal Botanic Gardens - Kew, Australian Plant DNA Bank and Trinity College Dublin (TCD) (Rice et al., 2006; Hodkinson et al., 2007). The Global Biodiversity Information Facility (GBIF) in Germany has establish a DNA Bank Network in 2007, last accessed 22 September 2010, and offers a worldwide central web portal, providing DNA samples of complementary collections (microorganisms, protists, plants, algae, fungi and animals). The GBIF Germany DNA network would provide a good mechanism to link both to the scientific community conserving genotypes in genebanks and to breeders and molecular biologists that use the resources for genetic improvement.

Pollen storage

Pollen storage was mainly developed as a tool for controlled pollination of asynchronous flowering genotypes, especially in fruit tree. Even if it may not be considered to be a viable method for meaningful genetic conservation of genotypes, cryopreservation is likely to be more successful than other storage techniques routinely employed for pollen. Pollen can be easily collected and cryopreserved in large quantities in a relatively small space. In addition, exchange of germplasm through pollen poses fewer quarantine problems compared with seed or other propagules.

The pollen longevity of different species varies between minutes and years depending on the taxonomic status of the plant and on abiotic environmental conditions. For some crops, the storage of pollen grains is possible in appropriate conditions, allowing their subsequent use for crossing with living plant material. It is also possible to regenerate haploid plants from pollen culture for some crops. By controlling the storage temperature and relative humidity (0 to 10°C, 10 to 30% RH, depending on species), pollens of *Citrus* spp*., Cocos nucifera, Fragaria* sp*., Olea europea, Pinus silvestris*, *Pistachio altantica, Pyrus malus* and *Vitis vinifera* could maintain their viability for more than 1 year.

For long-term conservation, cryopreservation seems to be the most efficient method. For example, maize pollen could be dried to 50% of its original water content in an air current for 1 h and then stored at -196ºC in liquid nitrogen. Deep-frozen maize pollen can be used for fertilization after 10 years storage. Successful cryopreservation of pollen from various 24 crops has been reported (Barnabas and Kovacs, 1997).

In situ conservation

In situ conservation refers to conservation of genetic resources within their ecosystem and natural habitats. These techniques involve maintenance of genetic variation at location where it is encountered, either in wild or traditional farming systems.

Genetic reserves: in this type of conservation location, management, and monitoring of genetic diversity is carried in natural wild populations within defined areas designated for active, long-term conservation.

On-farm conservation: This refers to the sustainable management of genetic diversity of locally developed traditional crop varieties with associated wild and weedy species or forms by farmers within traditional agricultural, horticultural or agrisilvicultural cultivation systems.

Home gardens: This type of conservation is similar to on-farm conservation, involves smaller scale but more species-diverse genetic conservation in home, kitchen, backyard or door-yard gardens.

Complementary conservation

For *ex situ* conservation of PGR in a crop or crop group, a gene pool approach has to be followed for safe and

effective conservation. Following this approach, it is very likely that a range of ex situ conservation methods would be applicable to satisfy the needs of a gene pool. For example, the rice gene pool consists of self-pollinated cultigens and a range of wild Oryza spp. habitat to range of climatic conditions with breeding ranging from obligate vegetative to facultative and obligate self-pollination. In a situation, it is quite logical to have an approach, which is appropriate and has balanced application of both in situ and ex situ conservation methods. This will lead to the adoption of a more “holistic” approach to conservation. Even with *ex situ*, a balance has to be struck as per the need. For example, in case of wild *Oryza* species, it has to be assessed, whether they would be best conserved in a field gene bank or *in vitro* as cell, tissue, organ, pollen or perhaps as DNA or in combination thereof. Therefore, a network of complementary and comprehensive strategy is needed to ensure effective conservation and sustainable use of PGR for food and agriculture by present and future generations.

Svalbard global seed vault

A major achievement for the conservation of the germplasm have been the creation of the of the Svalbard Global Seed Vault (SGSV) in 2008, established to serve as the ultimate safety net for seed samples from the world’s most important collections (GCDT, 2010). The Svalbard Global Seed Vault is a secure seedbank located on the Norwegian Island of Spitsbergen near the town of Longyearbyen in the remote Arctic Svalbard archipelago, about 1,300 km (810 miles) from the North Pole. The facility preserves a wide variety of plant seeds in an underground cavern. The seeds are duplicate samples, or "spare" copies, of seeds held in genebanks worldwide. The seed vault will provide insurance against the loss of seeds in genebanks, as well as a refuge for seeds in the case of large scale regional or global crises. The seed vault is managed under terms spelled out in a tripartite agreement between the Norwegian government, the Global Crop Diversity Trust (GCDT) and the Nordic Genetic Resource Center (also known as NordGen and previously named the Nordic Gene Bank, a cooperative effort of the Nordic countries under the Nordic Council of Ministers). The Prime Ministers of Norway, Sweden, Finland, Denmark, and Iceland participated in a ceremonial "laying of the first stone" on 19 June, 2006. The Svalbard Global Seed Vault opened officially on February 26, 2008. The first seeds arrived in January 2008. Five percent of the seeds in the Vault, about 18,000 samples with 500 seeds each, come from the Centre for Genetic Resources of the Netherlands (CGN), part of Wageningen University, Netherlands.

Construction of SGSV

The seedbank is constructed 120 m (390 ft) inside a sandstone mountain at Svalbard on Spitsbergen Island. The bank employs a number of robust security systems. Seeds are packaged in special four-ply packets and heat sealed to exclude moisture. The facility is managed by the Nordic Genetic Resource Center, though there is no permanent staff on-site.

Spitsbergen was considered ideal due to its lack of tectonic activity and its permafrost, which will aid preservation. The location 130 m (430 ft) above sea level will ensure that the site remains dry even if the icecaps melt. Locally mined coal provides power for refrigeration units that further cool the seeds to the internationally recommended standard -18°C (0°F). Even if the equipment fails, at least several weeks will elapse before the temperature rises to the -3°C (27°F) of the surrounding sandstone bedrock. Prior to construction, a feasibility study determined that the vault could preserve seeds from most major food crops for hundreds of years. Some seeds, including those of important grains, could survive far longer, possibly thousands of years.

Mission and seed storage

The Svalbard Global Seed Vault's mission is to provide a safety net against accidental loss of diversity in traditional genebanks. While the popular press has emphasized its possible utility in the event of a major regional or global catastrophe, it will certainly be more frequently accessed when gene banks lose samples due to mismanagement, accident, equipment failures, funding cuts and natural disasters. Such events occur with some regularity. In recent years, some national genebanks have also been destroyed by war and civil strife. There are some 1,400 *crop diversity collections* around the world, but many are in politically unstable or environmentally threatened nations. The seeds are stored in four-ply sealed envelopes, then placed into plastic tote containers on metal shelving racks. The storage rooms are kept at -18°C (-0°F). The low temperature and limited access to oxygen will ensure low metabolic activity and delay seed aging. The permafrost surrounding the facility will help maintain the low temperature of the seeds if the electricity supply should fail. Approximately 1.5 million distinct seed samples of agricultural crops are thought to exist. The variety and volume of seeds stored will depend on the number of countries participating – the facility has a capacity to conserve 4.5 million.

Gene bank standards

Research on seed storage has indicated that the potential of seeds to store, that is, retaining genetic integrity and seed viability, is influenced by storage seed moisture content and temperature. Germplasm is generally conserved as a base collection or an active collection. Base collections are those that are being

conserved on a long-term basis for posterity. These are unique accessions that are closest to the original samples and are not to be disturbed except for regeneration of active collections. Active or working collections are those that are immediately available for multiplication and distribution for use in research and crop improvement. To minimise the alteration in genetic structure and loss of viability in germplasm accessions during storage, the seed genebanks (that are part of the national network) preferably follow the genebank standards as recommended by FAO/IPGRI (Anonymous, 2001) in relation to various factors important to the good maintenance of active and base collections. The base collections are being stored in modules maintained at -20°C. Such a low temperature minimises metabolic activities and is expected to enable the seed to retain viability for 50 to 100 years without any change in genetic structure. Active collections are stored in modules maintained at 4°C and 35 to 40% relative humidity, under which seeds are expected to remain viable for 15 to 50 years without substantial change in viability and genetic integrity. For both types of collections, seed is processed after validating physical and genetic purity of seed, assessment of seed viability and seed moisture content. In most crops, seed samples with more than 85% seed viability are conserved. However, recognising inherent problems, such as indeterminate nature, which limits the harvest of physiologically mature seed of the same age in certain crops like cotton, several forages and vegetable crop species, the initial viability standards have been lowered down to between 50 to 75%. For long-term storage, the seed moisture content is brought down to 3 to 7%, while for medium-term storage the seed moisture content is brought down to 8 to 10%. For base collections to be put under long-term conservation, the preferable size of accession is 2,000 seeds in the case of self-pollinated and 4,000 in the case of cross-pollinated crops. However, in many cases, such as groundnut and castor, because of large seed size and low multiplication rates, the sample-size of the accessions has been reduced to between 1,000 to 1,500 seeds. The base and active collections are regularly monitored for seed viability, seed quantity, seed health, etc., at recommended intervals of 10 and 5 years, respectively. However, the monitoring of accessions at the National Seed Genebank (NSGB) in the Germplasm Conservation Division, NBPGR has generated valuable information on storability in a number of crop species, such as wheat, minor millets, cotton, grain legumes etc. (Anonymous, 2001). These results suggest a revision of the exact period of monitoring intervals. This information will be useful in revising the seed genebank standards in relation to other components and make seed conservation more cost effective. Seed storage problems are more common in India, because a large part of the country has a predominantly hot and humid, tropical and sub-tropical climate with great variation in temperature, rainfall and relative humidity across the year.

National network on conservation of PGR

Efficient conservation of PGR in a country of the size and dimension of India, one of the 12 mega-centres of plant biodiversity and where 384 crop plants are reported to be cultivated (of which 168 species were earlier reported under the Hindustani centre, one of the eight Vavilovian centres of origin and diversity (Paroda et al., 1999), essentially requires a network approach. Network facilitates short-, medium-, and long-term conservation requirements, the division of responsibilities, application of complementary conservation strategies, and access for the use of these genetic resources in crop improvement programmes. The national network consists of the NSGB at NBPGR headquarters, New Delhi, 11 NBPGR Regional Stations situated in different agro-climatic zones of the country, and 40 crop-based National Active Germplasm Sites (NAGS), located generally at various ICAR institutes. The network is linked with the All India Co-ordinated Crop Improvement Projects, various research institutes (crop-based institutes, project directorates and national research centres; multi-crop based institutes) in the ICAR, SAUs, etc. All network components operate in close collaboration to ensure the efficient conservation and sustainable use of germplasm in crop improvement, in which the National Seed Genebank plays a pivotal role in conservation.

The National Seed Genebank

The NSGB is responsible for conservation of seeds of unique accessions on a long-term basis, as base collections for posterity. In addition, it provides technical support to the network in the planning, development and operation of medium-term genebank facilities, in human resource development, and in provision of accessions for the regeneration of active collections. The Indian NSGB has 12 modules with a capacity to hold around 1 million accessions.

NBPGR Regional Stations

The NBPGR has 11 regional stations/base centres/satellite stations located in different agroecological and phytogeographical zones of the country. They are responsible for the collection, characterisation, evaluation and/or conservation of germplasm in the region. The regional stations also coordinate various PGR activities in the region with other partners. Seven of the regional stations have medium-term seed storage modules for the conservation of active collections to meet the requirement of the region for

various crops. The regional stations hold around 98,498 active collections. In addition, plant quarantine is looked after at the NBPGR headquarters, New Delhi and at the NBPGR regional station, Hyderabad.

National Active Germplasm Sites (NAGS)

The NAGS are based at ICAR institutes, at All India Co-ordinated Crop Improvement Projects and at SAUs. They are entrusted with the responsibility of crop specific collection, multiplication, evaluation, maintenance and conservation of active collections and their distribution to users at a national level. Large multiplications of active collections are preferred to reduce the number of regeneration cycles that can cause possible genetic changes and to meet the demand of seed distribution. The NAGS have a multidisciplinary team of scientists to study all the aspects of crop improvement, production and management. Therefore, the NAGS, in addition to their conservation role, are well equipped for the evaluation of germplasm and the generation of information on the potential value of accessions. This information forms the basis for use of accessions in research and crop improvement. Eleven of the NAGS have been provided with medium-term seed storage modules, to facilitate the use of active collections in research and breeding programmes.

Safety duplicates of crop species

There is a built-in duplicity of accessions in the system, wherein the accessions conserved at NAGS and the crop-based institutes as active collection are conserved as base collection in the National Genebank. The active collections are used in research and crop improvement and the National Genebank helps in restoration of lost accessions to the active sites. This also serves as safety mechanism. There exists medium to high capability for research and use of improved methodologies for *ex situ* conservation. Nevertheless, strengthening of technical and infrastructure capabilities is required in some cases.

The capacity building in genebank management and information systems has been carried out satisfactorily, though there is a need for extension of medium-term facilities to more crop based institutes to cover larger number of crops. In last ten years 196,745 accessions were collected under 166 projects involving 599 professional and of these 104 084 accessions have been conserved. The maximum number of accessions conserved in *ex situ* is in the category of traditional cultivars and landraces. A significant number of collections belonging to wild and weedy relatives and advanced and improved cultivars developed using various genetic resources is also being conserved.

Major achievements through germplasm conservation

Plant Genetic Resources for Food and Agriculture (PGRFA) are vital to the development and welfare of human society. They contribute enormously towards achieving the global objectives of food security and poverty alleviation, environment protection and sustainable development. The local communities and farmers in India have sustained and enriched the diversity of these resources which they domesticated, used, conserved and made available to meet the ever increasing needs of the present and future generations. Characterization and evaluation of germplasm is required to know its worth or usefulness and availability of information on characterization and evaluation of conserved genetic resources is the key to utilization. Plant breeding provides many examples of the use of genetic resources for the improvement of the varieties of crop plants. There are examples that range from highly specific improvement to one major factor such as susceptibility to a pest or disease to all round improvement in yield, agronomical traits, disease resistance and to changes in the form and structure of the plant type.

FUTURE THRUST

i) Endangered germplasm from the threatened areas of diversity to be salvaged and conserved for future use.
ii) Morphological and molecular characterization of germplasm to enhance their utilization in crop improvement.
iii) Conservation, management and protection of bio-resources especially plant resources, through the participation of the people.
iv) Conservation and use of diversity needed to be addressed in a holistic manner and to meet the demands of the users of germplasm.
v) Research on core and mini-core collections and identification of new diverse sources.
vi) Public awareness of the importance of CWR and neglected and underutilized species.
vii) Need to maximize synergy through appropriate collaboration between various national, sub-regional and international levels.

Conflict of Interest

The authors have not declared any conflict of interest.

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Scientific Research and Essays

Full Length Research Paper

Approximate solution to three point bending equation for a simply supported beam

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In this work, the authors present an approximate solution to three point bending equation for a simply supported beam of rectangular section. The deflection of the beam due to a perpendicular point – wise load is estimated for high rates of its curvature. After the deformation of the beam, the shape of its cross sectional area is assumed to remain as is. The proposed solution consists of binomial power series and does not contain elliptic integrals or other special functions. Hence, it may be more appropriate for usual engineering practices.

Key words: Simply supported beam, three point bending, large deflection.

INTRODUCTION

The three point bending test measures the force which is required to bend a beam under three point loading conditions. The data is often used to select materials for parts that will support loads without bending. Since the physical properties of many materials, (especially thermoplastics), can vary depending on ambient temperature it is sometimes appropriate to test materials at temperatures that simulate the intended end use environment. Bending tests are used for determining mechanical properties of various materials. Due to the important influence of shear effects in the displacements, great span-to-depth ratios are used in order to eliminate these effects. Three – point and four – point test configurations, are used to obtain flexural strength and flexural modulus. The rotation of the cross sections in the deformation process leads to the contact zone between specimen and cylindrical supports changing in a three-point bending test. The classical theory of deflection of beams neglects the square of the first derivative in the bending moment equation and cannot be used when the slopes of the beam are large. In many cases large deflections cannot be obtained without straining the beam plastically but, when the thickness of the beam is small compared with its width, large deflections within the elastic limit of the material are possible. Large deflection problems sometimes occur in some applications such as in the design of flat springs. Therefore, a general study on the large deflection behaviour of materials with high deformability is of great technological and practical importance. This problem has been dealt with by many researchers for a long time in relation to the buckling of members. From the investigation of the literature can be seen that the determination of the deflection curve of a simply supported or a cantilever beam subjected to various forms of loading traces back to Galileo who in 1638 had paused two problems concerning the construction of a cantilever. Over the following years these problems through the works of Hooke, Mariotte

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and Leibniz gradually yield to the question of the deflection curve of a cantilever. By 1691 the problem was narrowed by Bernoulli on columns and was finally solved in the general case by Euler in his famous treatise on elastic curves in 1743.

Upon the development of the theory of elliptic integrals and elliptic functions in the nineteenth century researchers sought to obtain a closed – form solution of the problem. The problem of a cantilever beam carrying a vertical concentrated load at its end has been recently solved approximately by Gross and Lehr (1938) and exactly by Barton (1945) and by Bishopp and Drucker (1945). Large deflection solution for the simply supported beam with a central concentrated load was given by Conway (1947) and by Frisch – Fay (1962) assuming the reaction support to be movable. Some formulas for beam columns were proved by Saelerman (1954). In all these cases, the equations are directly integrated leading to solutions expressible in terms of elliptic integrals. Rohde (1953) expanded the slope in a power series of arc length to study the large deflections of a cantilever with uniformly distributed load. The solution by the use of power series expansions for the large deflections of simply supported beams was studied by Iyengar and Rao (1955). The study of large deflection of a cantilever beam subjected to a concentrated load and a distributive one was performed by Lau (1981) whereas that on a cantilever mode of nonlinear materials subjected to a concentrated load by Lewis and Monasa (1981). Ohtsuki (1986) analysed the large deflection problem of a thin elastic simply supported beam for a symmetrical three – point bending, by solving the related nonlinear differential equation via analytical and numerical techniques. Also, bibliographies and review papers by Eisley (1963), Schmidt and Da Deppo (1971), Antman (1972) and Gorski (1976, 1979) indicated the continuing interest in this topic. A procedure enabling the numerically exact solution of the deflected shape was presented by Golley (1984). On the other hand, Timoshenko (1970) included the effect of the variation of the support span in the three-point test configuration due to the bending rotation at supports as a problem where the superposition principle is not applicable.

Theobald et al. (1977) carried out an experimental analysis in order to analyze the influence of load and geometric configuration in bending tests. The distance between loading noses or load span in a four-point bending test was varied in order to study the influence of this factor on the flexural strength and modulus. Experimental results showed that bending modulus varied in a significant manner when load span varied. Theocaris et al. (1977) investigated the three-point bending test at large deflections including friction forces at the supports, axial forces along the beam and the effect of span shortening due to roller supports. Hayat and Suliman (1998) performed tensile, three-point bending and four-point bending tests on glass reinforced phenolic laminates. Ogorkiewicz and Mucci (1971) carried out a large experimental investigation by examining a number of different methods of supporting specimens and in each case attempted to evaluate the deflections. Moreover, Mujika (2006) investigated the difference between flexural moduli obtained by three – point and four point bending tests.

In more recent publications, Tari (2013) investigated the parametric large deflections of Euler – Bernoulli cantilever beams, whereas in Mohyeddin and Fereidoon (2014) a research work is performed to analyze the large deflections of a straight prismatic shear – deformable beam resting on simple supports at both ends and subjected to a point – wise load at its midspan. Finally, in (Theotokoglou and Sideridis, 2011, 2014) analytical and numerical methods to predict the mechanical behaviour of composite beams in asymmetric four-point bending, were performed.

Analysis

Let us consider a rectangular simply supported continuum beam of span length L (Figure 1a, b) subjected in a point – wise perpendicular load of magnitude W at its midspan. This load causes an instant deflection δ, whose rate may increase with time due to creep phenomenon in the case of viscoelastic materials. Nevertheless we will centre our study only on the linearly elastic deformation.

Moreover, since this work constitutes an improvement to Freeman's method (Freeman, 1946), we additionally suppose that the displacements of the simple supports of the beam are high enough, such that the corresponding reactions to be perpendicular to the deformed beam. Besides, as we have already mentioned, though the deformation of the beam becomes significant its cross – sectional area remains rectangular. By considering the equilibrium equation, it follows

$$2X\cos a = W \tag{1}$$

The bending moment of an arbitrary point of the beam is

$$M = -\left(X \cdot x\cos a + X \cdot y\sin a\right) \tag{2}$$

On the other hand, according to the Theory of Elasticity the following equation arises

$$M = \frac{EI}{R} \tag{3}$$

Where E denotes Young Modulus, I the moment of inertia and R the radius of the local curvature of the beam at this point. According to elementary differential geometry the local curvature at any point of the graph of

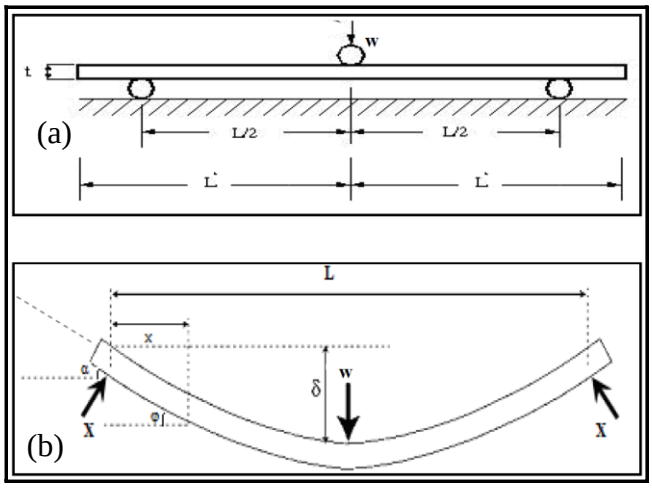

Figure 1a, b. Loading and deformation of the beam.

a single – valued function $y = y(x)$ is given as:

$$k = \left| \frac{\frac{d^2 y}{dx^2}}{\left(1 + \left(\frac{dy}{dx}\right)^2\right)^{\frac{3}{2}}} \right| \quad (4)$$

From Figure 1b we deduce

$$\tan\phi = \frac{dy}{dx} \quad (5)$$

and

$$\frac{ds}{dx} = \left|1 + \left(\frac{dy}{dx}\right)^2\right|^{1/2} \quad (6)$$

Where s denotes an arbitrary element of length, measured from the origin of the beam.
Apparently, the following relationships hold

$$R = \frac{ds}{d\phi} \quad (7)$$

$$\cos\phi = \frac{dx}{ds} \quad (8)$$

$$\sin\phi = \frac{dy}{ds} \quad (9)$$

A combination of Equation (2) and (3) yields

$$\frac{EI}{R} = -\left(X \cdot x\cos a + X \cdot y \sin a\right) \quad (10)$$

Then, by differentiating Equation (10) with respect to the variable s and taking also into account Equations (8) and (9) one obtains (Freeman, 1946).

$$-\frac{EI}{R^2} \cdot \frac{dR}{ds} = -\left(X\cos a\ \cos\phi + X \sin a\ \sin\phi\right) \Leftrightarrow$$

$$\frac{EI}{R^3} \cdot \frac{dR}{d\phi} = X\cos(a - \phi) \quad (11)$$

An integration of Equation (11) with respect to the variable ϕ implies

$$-\frac{EI}{2R^2} = -X\sin(a - \phi) + C_1 \quad (12)$$

When $M = 0$ it follows that $\phi = a$ and $\frac{1}{R} \to 0$, therefore we infer that $C_1 = 0$

Hence, by solving Equation (12) for $\frac{1}{R}$ we arrive at the following expression

$$\frac{1}{R} = \left|\frac{2X}{EI}\sin(a - \phi)\right|^{1/2} \quad (13)$$

Finally, Equation (13) in combination with Equations (7), (8) and (9) results in the following system (Freeman, 1946).

$$x=\left(\frac{EI}{2X}\right)^{1/2}\cdot\int_{a}^{\phi}\cos\phi\cdot\frac{1}{\sqrt{\sin(a-\phi)}}d\phi \quad (14a)$$

$$y=\left(\frac{EI}{2X}\right)^{1/2}\cdot\int_{a}^{\phi}\sin\phi\cdot\frac{1}{\sqrt{\sin(a-\phi)}}d\phi \quad (14b)$$

Next, to estimate the deflection due to the point – wise loading of magnitude W imposed at the midspan of the beam, we apply the following boundary conditions:

$$\phi=0\Rightarrow y=\delta \quad (15a)$$

$$\phi=0\Rightarrow x=\frac{L}{2};X=\frac{W}{2\cos a} \quad (15b)$$

Consequently, the system Equation (14a) and (14b) combined with Equation (15a) and (15b) yields

$$\frac{1}{2}\cdot\left(\frac{WL^2}{EI}\cdot\frac{1}{\cos a}\right)^{1/2}=-\int_{0}^{a}\cos\phi\cdot\frac{1}{\sqrt{\sin(a-\phi)}}d\phi \quad (16a)$$

$$\frac{\delta}{L}\cdot\left(\frac{WL^2}{EI}\cdot\frac{1}{\cos a}\right)^{1/2}=-\int_{0}^{a}\sin\phi\cdot\frac{1}{\sqrt{\sin(a-\phi)}}d\phi \quad (16b)$$

Considering the angle a as a parameter, the fraction $\frac{WL^2}{EI}$ can be evaluated from Equation (16a) given an exact or approximate calculation of the integral $I_a=-\int_{0}^{a}\cos\phi\cdot\frac{1}{\sqrt{\sin(a-\phi)}}d\phi$.

Evidently, Equation (16b) enables us to represent the fraction $\frac{\delta}{L}$ in terms of the fraction $\frac{WL^2}{EI}$.

In the sequel, if one sets $a-\phi=\omega,\ \omega\in R$ the following relationships arise

$$\phi=a-\omega;\ d\phi=-d\omega;\ \cos\phi=\cos(a-\omega);\ \sin\phi=\sin(a-\omega)$$

Hence we infer

$$I_a=+\int_{a}^{0}\cos(a-\omega)\cdot\frac{1}{\sqrt{\sin\omega}}d\omega\Rightarrow$$

$$I_a=-\int_{0}^{a}\cos(a-\omega)\cdot\frac{1}{\sqrt{\sin\omega}}d\omega \quad (17a)$$

and

$$I_b=+\int_{a}^{0}\sin(a-\omega)\cdot\frac{1}{\sqrt{\sin\omega}}d\omega$$

or equivalently

$$I_b=-\int_{0}^{a}\sin(a-\omega)\cdot\frac{1}{\sqrt{\sin\omega}}d\omega \quad (17b)$$

Then Equation (17a) yields

$$I_a=-\int_{0}^{a}\cos a\cos\omega\cdot\frac{1}{\sqrt{\sin\omega}}d\omega+\int_{0}^{a}\sin a\cdot\sqrt{\sin\omega}\,d\omega \quad (17c)$$

The latter can be recasted to give

$$I_a=-\cos a\int_{0}^{a}\cos\omega\cdot\frac{1}{\sqrt{\sin\omega}}d\omega+\sin a\int_{0}^{a}\sqrt{\sin\omega}\,d\omega\Leftrightarrow$$

$$I_a=-\cos a\int_{0}^{a}\frac{2\cos\omega}{2\sqrt{\sin\omega}}d\omega+\sin a\int_{0}^{a}\sqrt{\sin\omega}\,d\omega \quad (17d)$$

After the necessary algebraic manipulation the following expression arises

$$I_a=-2\cos a\sqrt{\sin a}+\sin a\int_{0}^{a}\sqrt{\sin\omega}\,d\omega \quad (18a)$$

Similarly, Equation (17b) yields

$$I_b=-\int_{0}^{a}\sin a\cos\omega\frac{1}{\sqrt{\sin\omega}}d\omega+\int_{0}^{a}\cos a\sqrt{\sin\omega}\,d\omega \quad (18b)$$

The latter equation can be formulated as follows

$$I_b=-\sin a\int_{0}^{a}\cos\omega\frac{1}{\sqrt{\sin\omega}}d\omega+\int_{0}^{a}\cos a\sqrt{\sin\omega}\,d\omega\Leftrightarrow$$

$$I_b=-\sin a\int_{0}^{a}\frac{2\cos\omega}{2\sqrt{\sin\omega}}d\omega+\int_{0}^{a}\cos a\sqrt{\sin\omega}\,d\omega \quad (18c)$$

After some algebra, the following expression emerges

$$I_{\mathrm{b}}=-2\sin a\sqrt{\sin a}+\cos a\int_0^a\sqrt{\sin\omega}\,d\omega \tag{18d}$$

Hence, to derive explicit representations for the terms I_{a} and I_{b} it is necessary the obtaining of a closed form expression of the quantity $I_c=\int_0^a\sqrt{\sin\omega}\,d\omega$ or generally the estimation of the antiderivatives of the continuous single valued function $\sqrt{\sin\omega}$, i.e. the set $\int\sqrt{\sin\omega}\,d\omega$. To this end, by making the substitution $t=\sqrt{\sin\omega}$ it follows

$$dt=\frac{\cos\omega}{2\sqrt{\sin\omega}}d\omega\Rightarrow\ d\omega=\frac{2\sqrt{\sin\omega}}{\cos\omega}dt\Rightarrow$$
$$d\omega=\frac{2t}{\sqrt{1-t^4}}dt \tag{19}$$

and therefore

$$I_c=2\int_0^{\sqrt{\sin a}}\frac{t^2}{\sqrt{1-t^4}}dt \tag{20}$$

Hence, we have arrived at a binomial integral whose irrational term is in the denominator of the fraction. Primarily, if we concentrate on this binomial indefinite integral we can point out that none of the three Chebyschev's Conditions (Nikolsky 1977), which would permit us to analyze it in trivial functions of Elementary Calculus, is satisfied. Indeed, since any binomial integral $I=x^m\left(\mathrm{ax}^{\mathrm{n}}+b\right)^k\ \forall m,n,k\in Q$ can be represented in terms of elementary single – valued continuous functions if and only if the following statement holds (Nikolsky 1977).

$$k\in Z\ \vee\ \frac{m+1}{n}\in Z\ \vee\ \frac{m+1}{n}+k\in Z \tag{21 a,b,c}$$

However, by taking into consideration that the single – valued function of the generic form $f(x)=(1+x)^k\ \forall x\in R,\ \forall k\in Q$ is analytic over the interval $(-1,+1)$, where Cauchy residue of its corresponding MacLaurin expansion tends to zero, one can also expand the quantity $\frac{1}{\sqrt{1-t^4}}$ in powers of the variable t, as a binomial series. Thus, it can be written that

$$\frac{1}{\sqrt{1-t^4}}=1+\frac{1}{2}t^4+\frac{1\cdot3}{2\cdot4}t^8+\frac{1\cdot3\cdot5}{2\cdot4\cdot6}t^{12}+\ldots+\frac{1\cdot3\cdot5\cdot\ldots\cdot(2n-1)}{2\cdot4\cdot6\cdot\ldots\cdot2n}t^{4n} \tag{22}$$

Hence we infer

$$\frac{t^2}{\sqrt{1-t^4}}=t^2+\frac{1}{2}t^6+\frac{1\cdot3}{2\cdot4}t^{10}+\frac{1\cdot3\cdot5}{2\cdot4\cdot6}t^{14}+\ldots+\frac{1\cdot3\cdot5\cdot\ldots\cdot(2n-1)}{2\cdot4\cdot6\cdot\ldots\cdot2n}t^{4n+2} \tag{23}$$

Consequently, one can calculate the indefinite integral $\int\frac{t^2}{\sqrt{1-t^4}}dt$ via a term by term integration of Equation (23) with respect to the variable t.

$$\int\frac{t^2}{\sqrt{1-t^4}}dt=\int\left[t^2+\frac{1}{2}t^6+\frac{1\cdot3}{2\cdot4}t^{10}+\frac{1\cdot3\cdot5}{2\cdot4\cdot6}t^{14}+\ldots+\frac{1\cdot3\cdot5\cdot\ldots\cdot(2n-1)}{2\cdot4\cdot6\cdot\ldots\cdot2n}t^{4n+2}\right]dt$$
$$\Rightarrow$$
$$\int\frac{t^2}{\sqrt{1-t^4}}dt\cong\frac{t^3}{3}+\frac{t^7}{14}+\frac{3t^{11}}{88} \tag{24}$$

with $t=\sqrt{\sin\omega}$

Here, we can also elucidate that since the quantity $\sqrt{\sin\omega}$ does not appear in the denominator of any fraction of the above representation, none of the terms of this summation will tend to infinity when the variable ω becomes zero.

Thus, by returning to Equation (20) we approximately estimate the integral I_c as follows

$$I_c\cong\left[\frac{2t^3}{3}+\frac{t^7}{7}+\frac{3t^{11}}{44}\right]_0^{\sqrt{\sin a}} \tag{25}$$

Therefore, Equation (18a) and (18d) respectively are now written out as follows:

$$I_{\mathrm{a}}=-2\cos a\ \sqrt{\sin a}+\sin a\left[\frac{2t^3}{3}+\frac{t^7}{7}+\frac{3t^{11}}{44}\right]_0^{\sqrt{\sin a}} \tag{26a}$$

$$I_{\mathrm{b}}=-2\sin a\sqrt{\sin a}+\cos a\left[\frac{2t^3}{3}+\frac{t^7}{7}+\frac{3t^{11}}{44}\right]_0^{\sqrt{\sin a}} \tag{26b}$$

Hence, Equation (16a) and (16b) in association with Equation (26a) and (26b) yield

$$\frac{1}{2}\cdot\left(\frac{WL^2}{EI}\cdot\frac{1}{\cos a}\right)^{1/2}=-2\cos a\ \sqrt{\sin a}+\sin a\frac{2(\sqrt{\sin a})^3}{3}+\frac{\sin a(\sqrt{\sin a})^7}{7}+\frac{3\sin a(\sqrt{\sin a})^{11}}{44} \tag{27a}$$

and

$$\frac{\delta}{L}\cdot\left(\frac{WL^2}{EI}\cdot\frac{1}{\cos a}\right)^{1/2}=-2\sin a\sqrt{\sin a}+\cos a\frac{2(\sqrt{\sin a})^3}{3}+\frac{\cos a(\sqrt{\sin a})^7}{7}+\frac{3\cos a(\sqrt{\sin a})^{11}}{44} \quad (27b)$$

Thus, according to the above mathematical procedure the fraction $\frac{\delta}{L}$ is now represented in terms of the fraction $\frac{WL^2}{EI}$ for any possible rate of the angle a. Here, we emphasize that according to the symmetry of the physical problem we study, that is, location of frictionless supports and load, the single – valued function which describes the deflection should have a global maximum at the midspan of the beam. In addition, an other interesting conclusion arising from the above fundamental result has been illustrated in the following figure, where one can deduce by intuition that to the unique circle which is defined by the chord AB of length L, that is, the simply supported beam before its elastic deformation and the point M of its maximum deflection, there corresponds a tangential segment having minimum value, when the maximum rate of the deflection occurs in the midspam of the elastically deformed beam. Apparently, the length ΓΔ of this aforementioned segment represents the influence of the point – wise loading to the overall deformation of the beam. Nevertheless, a rigorous mathematical interpretation of this observation is performed below (Figure 2). Let us set ΟΔ = x_0, ΟΓ = y_0 and ΓΔ = ω. Then, from Pythagorean Theorem it follows

$$x_0^2+y_0^2=d^2 \quad (28)$$

Since ΓΑ=ΓΜ and ΟΑ = ΟΒ = R it implies that

$$x_0+y_0+d=2\mathrm{R}\Rightarrow y_0=2\mathrm{R}-x_0-d \quad (29)$$

and therefore

$$y_0^2=4\mathrm{R}^2+x_0^2+d^2+2(x_0d-2Rx_0-2Rd) \quad (30)$$

A combination of Equations (29) and (30) yields

$$d^2=x_0^2+4R^2+x_0^2+d^2+2(x_0d-2R-2Rd)\Leftrightarrow$$
$$x_0^2-(2R-d)\,x+x_0^2+2R(R-d)=0 \quad (31a)$$

Since x_0 is be a real number the discriminant of the above quadratic equation should be nonnegative. Hence, we deduce that

$$d^2+4R\mathrm{d}-4R^2\geq 0 \quad (31b)$$

The roots of the above quadratic function are $2R(-1-\sqrt{2})$ and $2R(\sqrt{2}-1)$

Evidently, the latter inequality holds if and only if

$$d\leq 2R(-1-\sqrt{2}) \quad (32a)$$
$$\vee$$
$$d\geq 2R(\sqrt{2}-1) \quad (32b)$$

Since it is obvious that the variable d should take strictly positive values, we infer that Equation (32a) cannot hold. Thus it implies

$$\min\{d\}=2R(\sqrt{2}-1) \quad (33)$$

Hence, by introducing Equation (33) into (31a) one obtains

$$x_0^2-2R\cdot(2-\sqrt{2})\,x+6R^2-4R^2\sqrt{2}=0 \quad (34)$$

The solution procedure of the above quadratic equation for x_0 yields

$$x_0=\frac{2R(2-\sqrt{2})\pm\sqrt{4R^2(2-\sqrt{2})^2-4R^2(6-4\sqrt{2})}}{2}\Leftrightarrow$$
$$x_0=R(2-\sqrt{2}) \quad (35)$$

Hence Equations (29), (33) and (35) can be combined to yield the following disjunction

$$y_0=x_0 \quad (36)$$
$$\vee$$
ΟΔ = ΟΓ (37)

Therefore, when the point M lays in the middle of the arc AOB (Figure 2) the influence of the point – wise loading to the overall deformation of the beam is minimum.

Next, referring to the equation of elastic curve on the beam which obviously has the general implicit form $f(x,y)=0$ it is known from multi – valued Calculus (Hilbebrandt, 1976) that for every implicit function of such a form, the following identity holds:

$$\left(\frac{dx}{dy}\right)^2\cdot\frac{d^2y}{dx^2}+\frac{dy}{dx}\cdot\frac{d^2x}{dy^2}\equiv 0 \quad (38)$$

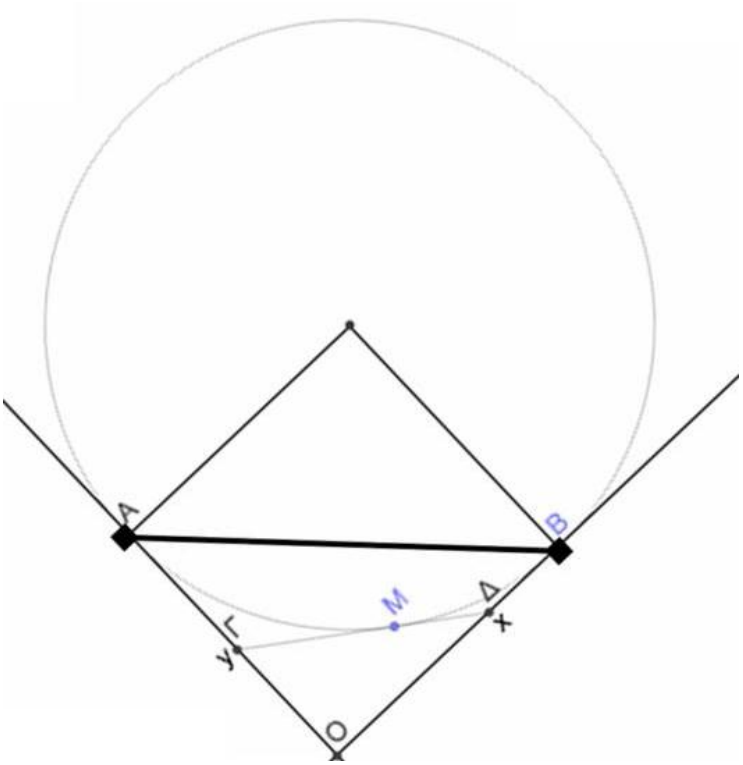

Figure 2. Qualitative localization of the maximum deflection.

The above relationship in association with Equations (4) and (6) introduces a first restriction for the local curvature of the beam, provided that the implicit function $f(x,y)=0$ is invertible over the interval [0, L].

On the other hand, it is also known from analytic geometry (Riddle, 1995) that if $(x_1,y_1),(x_2,y_2)$ are the endpoints of the beam, with $\sqrt{(x_1-x_2)^2+(y_1-y_2)^2}=L$, then the circle which is univocally defined has the following equation

$$(x-x_1)\cdot(x-x_2)+(y-y_1)\cdot(y-y_2)=0 \quad \Leftrightarrow$$

$$x^2-(x_1+x_2)\cdot x+x_1\cdot x_2+$$
$$y^2-(y_1+y_2)\cdot y+y_1\cdot y_2=0 \tag{39}$$

To obtain the derivative $y'(x)$ of the above implicit relationship we consider that the left member of this expression is identically zero. So, we can write out

$$2x-(x_1+x_2)+2y\cdot y'(x)-(y_1+y_2)=0 \tag{40}$$

Hence, by solving the latter equation for $y'(x)$ one finds

$$y'(x)=\frac{(y_1+y_2)+(x_1+x_2)-2x}{2y} \tag{41}$$

Then, the angle θ between the tangent lines of the above circle and the implicit relationship $f(x,y)=0$ of the curve line of the beam at their intersection point (x_a,y_a) is given by the following formula

$$\tan\theta=\frac{\frac{dy_a}{dx_a}-\frac{(y_1+y_2)+(x_1+x_2)-2x_a}{2y_a}}{1+\frac{dy_a}{dx_a}\cdot\frac{(y_1+y_2)+(x_1+x_2)-2x_a}{2y_a}} \tag{42}$$

Thus, we infer that when $x_a=\frac{x_1+x_2}{2}$ and $\theta=\kappa\pi+\frac{\pi}{2}$, that is, the value of $\tan\theta$ tends to infinity, then y_a coincides with the maximum deflection of the beam.

DISCUSSION

An approximate solution to three point bending equation for a simply supported continuum beam of rectangular

section area was presented. The deflection of the beam due to a point – wise load was evaluated for high rates of its curvature. However, in this study we have not considered that the points of the beam in contact with the supports are at their upper surface. Also, it has been observed that for large deflections of the beam the reactions at the supports will not be quite perpendicular. Therefore, to make the problem as general as possible the forces at the supports should be assumed to consist of perpendicular reactions X and tangential forces $T=\mu X$, where μ is the coefficient of friction between beam and supports.

In these cases, referring to the angles of the reactions due to frictionless supports at the endpoints $(x_1,y_1),(x_2,y_2)$ of the beam, Equation (42) yields respectively

$$\tan\theta_1=\frac{\frac{dy_1}{dx_1}-\frac{(y_1+y_2)+(x_1+x_2)-2x_1}{2y_1}}{1+\frac{dy_1}{dx_1}\cdot\frac{(y_1+y_2)+(x_1+x_2)-2x_1}{2y_1}}$$

$$\Leftrightarrow \tan\theta_1=\frac{\frac{dy_1}{dx_1}-\frac{y_1+y_2+x_2-x_1}{2y_1}}{1+\frac{dy_1}{dx_1}\cdot\frac{y_1+y_2+x_2-x_1}{2y_1}}$$

Since $y_1\equiv y_2$ it follows

$$\tan\theta_1=\frac{\frac{dy_1}{dx_1}-1+\frac{x_2-x_1}{2y_1}}{1+\frac{dy_1}{dx_1}\cdot\left(1+\frac{x_2-x_1}{2y_1}\right)}$$

Also,

$$\tan\theta_2=\frac{\frac{dy_2}{dx_2}-\frac{(y_1+y_2)+(x_1+x_2)-2x_2}{2y_2}}{1+\frac{dy_2}{dx_2}\cdot\frac{(y_1+y_2)+(x_1+x_2)-2x_2}{2y_2}}$$

$$\Leftrightarrow \tan\theta_2=\frac{\frac{dy_2}{dx_2}-\frac{y_1+y_2+x_1-x_2}{2y_2}}{1+\frac{dy_2}{dx_2}\cdot\frac{y_1+y_2+x_1-x_2}{2y_2}}$$

Since $y_1\equiv y_2$ it follows

$$\tan\theta_2=\frac{\frac{dy_2}{dx_2}-1+\frac{x_1-x_2}{2y_2}}{1+\frac{dy_2}{dx_2}\cdot\left(1+\frac{x_1-x_2}{2y_2}\right)}$$

Obviously, these equations constitute a constraint for the angles between the beam and the reactions of its frictionless supports at the endpoints of the beam.

Moreover, when $\theta_1=\kappa\pi+\frac{\pi}{2}$ or $\theta_2=\kappa\pi+\frac{\pi}{2}$, the denominators of the above quotients should vanish and therefore

$$\frac{dy_1}{dx_1}\cdot\left(1+\frac{x_2-x_1}{2y_1}\right)=-1\Leftrightarrow\frac{dy_1}{dx_1}=-\frac{2y_1}{2y_1+x_2-x_1}$$

$$\frac{dy_2}{dx_2}\cdot\left(1+\frac{x_1-x_2}{2y_2}\right)=-1\Leftrightarrow\frac{dy_2}{dx_2}=-\frac{2y_2}{2y_2+x_2-x_1}$$

In addition, in large deflections the beam sections are stressed not only by the bending moment but also by an axial force which is developed. This force creates a displacement of the neutral axis thus the stiffness of the beam does not remain invariant.

Conclusions

In this investigation, we were concerned with the large deflections of simply supported continuum beams. It was observed that the maximum deflection of a beam carrying a central concentrated load, that is, when submitted in three point bending, was different than that predicted by the simple bending theory due to a number of variables that should be considered. However, an appropriate choice can reduce the effect of these variables on the results of the tests. The load – deflection curve is not linear but deflection variations increase with corresponding increments of load. Also, it was estimated that when the reactions are assumed perpendicular to the beam there is a lateral component tending to change the deflection and slope since the buckling component of the perpendicular reactions becomes predominant.

Conflict of Interest

The authors have not declared any conflict of interest.

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